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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

PHYSIOLOGY

REFLEX EFFECTS ON THE HEART FROM THE PROPRIOCEPTORS

ALTERATION OF CONDITIONED REFLEXES FORMED ON A BASIS OF UNCONDITIONED REFLEX EFFECTS ON THE HEART FROM THE PROPRIOCEPTORS

I. A. Sapov

From the Naval Medical Academy, Leningrad

(Received June 5, 1956. Presented by Academician K. M. Bykov)

In earlier articles [5, 6], we presented data concerning the speed of the formation, elimination and restoration of positive conditioned reflexes, the development of which was based on unconditioned reflex effects on the heart from the proprioceptors, and on the development of a differentiation. This article will present the results obtained by changing the signal meaning of the conditioned stimuli. This series of experiments was conducted on five dogs.

We should mention that certain peculiarities in the reactions of two dogs, Jack and Treasure, to conditioned stimuli and to an unconditioned stimulus developed before these experiments were conducted. After many uses of the differentiation stimulus, the negative conditioned stimulus, a metronome of 60 beats per minute (M60) caused no change in heart activity. The positive conditioned stimulus, a metronome of 120 beats per minute (M120), after being combined many times with a static physical load, caused no change in heart activity either. There was a fourth phase observed in the reaction of the animals to both the unconditioned stimulus and to the unconditioned and conditioned stimuli combined. At this time, the stimuli M120 and M60, as well as the static load, seemed to have no effect on heart activity. The question was – was the heart activity of the animals really unaffected by these stimuli?

The experiments with the alteration of the conditioned reflexes answered this question. Considerable changes occurred in heart activity during reinforcement of the differentiation stimulus by the unconditioned; they were expressed by changes in all elements of the electrocardiogram. The negative T wave became isoelectric or positive (most pronounced in the dog Treasure), the number of heart contractions increased considerably, and respiratory arrhythmia disappeared (most pronounced in the dog Jack).

The alteration of the conditioned reflexes was virtually perfected during Experiment I after 4 combinations in Treasure and after 3 in Jack. On the following day, M60 used alone in the beginning of the experiment caused the same changes observed when it was combined with the unconditioned stimulus. M120, which had been used twice without reinforcement the preceding day, caused no evident change in heart activity, just as before the alteration of the conditioned reflexes. We continued to reinforce the altered conditioned reflexes for 3 more days in Treasure and for 2 more in Jack. At this time, the earlier differentiation stimulus, M60, reproduced the changes which it caused during its first combinations with the unconditioned stimulus.

The changes which occurred in the alteration process became permanent and were observed without the use of the stimuli beginning with the second day of conditioned reflex signal meaning alteration in Jack and with the third day in Treasure. Moreover, the unconditioned stimulus caused no change in heart activity, which remained disturbed to the same extent as before. The new positive stimulus (formerly the differentiation) intensified these changes. For example, in the dog Jack, only the rhythm of the heart activity changed when M60 was combined with the static load, while the use of M60 alone caused not only a more pronounced rhythm acceleration, but also,

in separate instances, caused the negative T wave of the electrocardiogram to be transformed into a positive wave. The former positive stimulus, which ceased to be reinforced when conditioned reflex alteration began, normalized the heart activity. The above is illustrated in the graphs of Jack's heart activity rhythm (Fig. 1) and in the electrocardiograms of Treasure (Fig. 2). After conditioned reflex alteration began, Treasure's outward behavior was about the same, but Jack showed a general motor restlessness, pulling away from the bench and gnawing at the straps.

Since the changes in the heart activity of these animals became stable 2-3 days after conditioned reflex alteration was begun, further action of the stimuli was stopped. The dogs were allowed to rest, during which time we made periodic electrocardiographic observations until the original indices of heart activity were restored. The animals were placed on the benches for 15-20 minutes, and electrocardiograms in the 3 standard leads were recorded. The observations were not conducted in the conditioned reflex chamber, in which the alteration of the stimulus signal meaning had been done, but in an ordinary laboratory room. Here, for the first few days, we noted changes in the animals' heart activity identical to those observed in the conditioned reflex chamber.

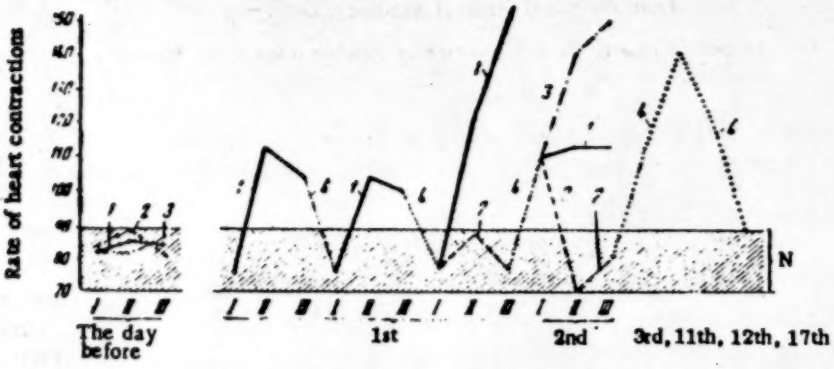

Fig. 1. Dynamics of the changes in the heart activity rhythm of the dog Jack during conditioned reflex alteration.
I) Background; II) during the action of the stimuli; III) after the action of the stimuli; on the 1st, 2nd, 3rd, 11th, 12th and 17th days after alteration began; 1) M60 combined with unconditioned stimulus, but static load alone on the day before alteration and on the 2nd day afterward; 2) M120; 3) M60; 4) undetected rhythm changes; N – normal range of rhythm.

Periodic electrocardiographic study of the animals showed heart activity approximated the original level in the dog Treasure 14 days after alteration began (Fig. 2, see Fig. 3, electrocardiographic forms I, II, III of this dog before the conditioned reflex experiments), and, in the dog Jack, heart activity became normalized 17 days after the beginning of conditioned reflex alteration (Fig. 1).

After the electrocardiogram indices had returned to the original level, which was observed in conditions of an ordinary laboratory room, the dogs were not used again experimentally for more than three months: Treasure was used after 95 days, Jack after 96. Then observations on them were continued. When Jack was first used in the chamber after the recess, his electrocardiogram showed no change from the original (normal) both without the use of the stimuli (3 experiments) and with their use (4 experiments). However, when Treasure was used in the conditioned reflex chamber, changes appeared in the electrocardiogram which were analogous to those caused by the alteration of the conditioned stimuli signal meanings: there was some increase in the number of heart contractions as compared with the original background, but the principal change was a disturbance in the end section of the electrocardiogram; a positive T wave was observed instead of the negative normally observed.

These electrocardiographic changes were observed in the conditioned reflex chamber without the use of stimuli (4 experiments). As before the recess, M60 intensified heart activity disturbance in this dog, while M120

almost completely normalized it (5 experiments). Moreover, the electrocardiogram recorded of Treasure during this period in the laboratory room, where the electrocardiographic observations were conducted, immediately after the conditioned reflex alteration was hardly distinguishable from the normal. This led to the proposal that changes in heart activity caused by the conditioned reflex alteration were due to the whole complex of experimental conditions as well as to the specific use of the stimuli.

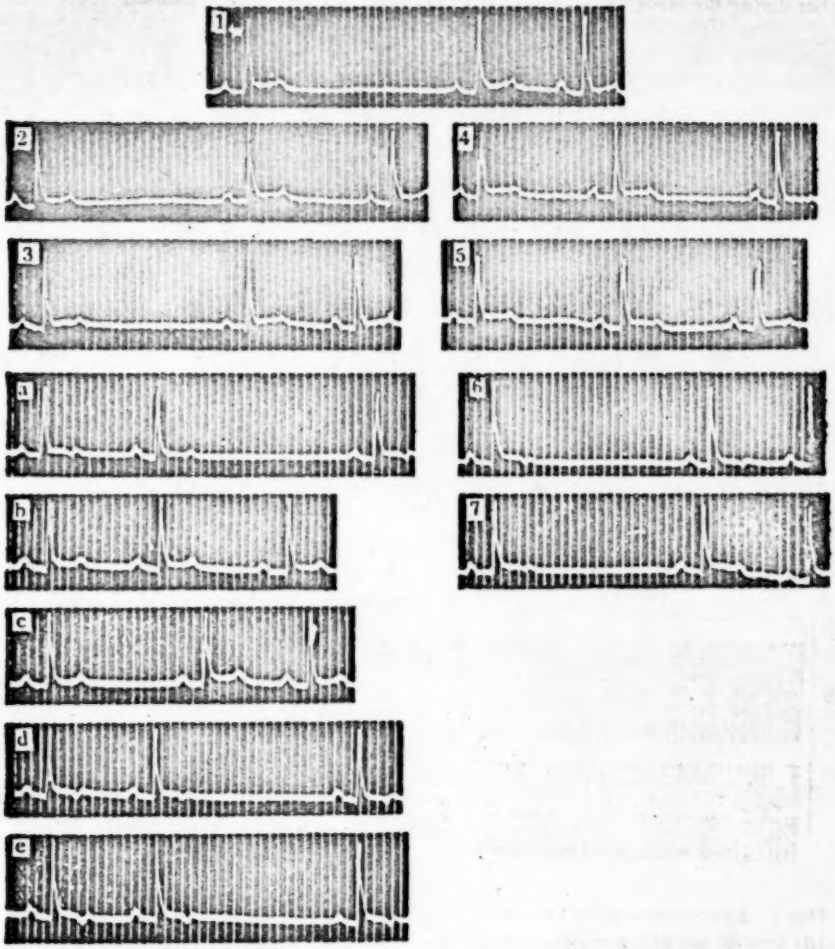

Fig. 2. Electrocardiograms showing the changes in the heart activity of the dog Treasure caused by the conditioned reflex alteration (the II leads).
1-7) On the 4th day after alteration began; 1) background; 2) during the action of the unconditioned stimulus; 3) after the action of the unconditioned stimulus; 4) during the action of M60; 5) after the action of M60; 6) during the action of M120; 7) after the action of M120; on the 10th (a), 11th (b), 12th (c), 14th (d) and 17th (e) days after the beginning of conditioned reflex alteration.

Specific experiments were conducted to prove this proposal: electrocardiograms of the animal were first recorded in the conditioned reflex chamber and then, 15-20 minutes later, in the laboratory room, and then the process was repeated inversely. Figure 3 shows the electrocardiograms obtained from Treasure in two such experiments; they show that the setting in which the signal meaning of the stimuli had been altered (the conditioned

reflex chamber) seemed to have become a "pathologic stimulus", while the removal of the animal from these surroundings caused the electrocardiographic indices to approximate the original and heart activity to be normalized.

When the dog Treasure was placed systematically over a period of a month in the conditioned reflex chamber for 30-40 minutes a day (22 experiments), the electrocardiograms recorded during this time showed the gradual return to normal of the heart activity, but slight electrocardiographic changes were observed periodically even after this. Electrocardiograms recorded from the dog Jack in the laboratory room and in the conditioned reflex chamber during the same experiment were indistinguishable from each other.

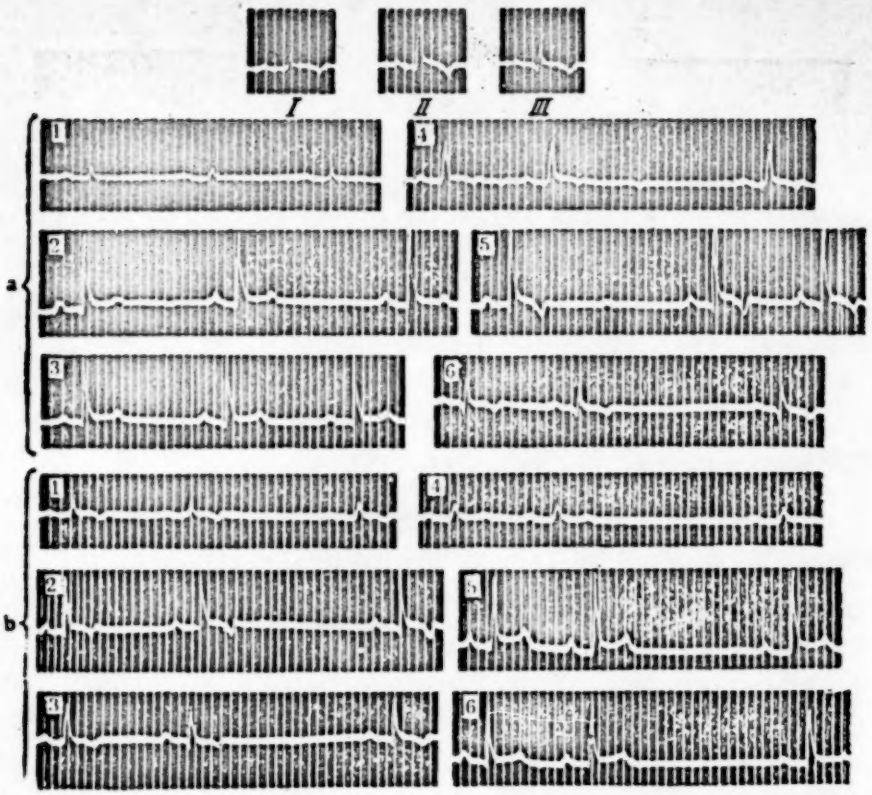

Fig. 3. Electrocardiograms recorded from the dog Treasure in different rooms. I, II and III) form of the electrocardiogram in leads from the extremities before the formation of the conditioned reflexes was begun; 1, 2, 3 (a)) electrocardiograms in the conditioned reflex chamber, in order – leads I, II and III; 4, 5, 6 (a)) the same in the laboratory room 15 minutes later; 1, 2, 3 (b)) electrocardiograms in the laboratory room, leads I, II and III; 4, 5, 6 (b)) the same 15 minutes later in the conditioned reflex chamber.

Therefore, in the fourth phase of training to the static load, the alteration of the proprioceptive conditioned reflexes on the heart was quickly accomplished. Three to four combinations of the differentiation stimulus with the unconditioned stimulus sufficed. However, higher nervous activity in the animals was disrupted due to the alteration of the proprioceptive conditioned reflexes. This neurotic condition was expressed in heart activity disturbances which differed as to character and duration depending on the dog. This would seem to be primarily connected with the experimental conditions and with the type of higher nervous activity in the animals, as well as with their age. When the signal meaning of the stimuli was altered, only a persistent change in the rate of

the heart contractions occurred in the young dog, Jack (aged about 3 years). Only 17 days after alteration of the signal meaning of the stimuli was begun did the heart rhythm return to normal and the animal finally emerge from the neurotic condition. In the old dog, Treasure (about 9 years old), the neurotic condition was characterized by a distorted form of stimulation stoppage in the ventricular myocardium or by a disturbance of the restoration processes in the cardiac muscle [1, 2, 3, 4 and others], as well as by change in the heart activity rate. When this animal was removed from the setting in which the conditioned reflexes had been altered, heart activity was normalized; the entire complex of experimental conditions had become a "pathologic" stimulus which could cause a neurotic condition even 5 months after the beginning of stimuli signal meaning alteration.

One must again emphasize that although the use of the new positive stimulus (formerly the differentiation) aggravated the heart activity disturbances throughout the neurotic period, the use of the former positive stimulus (now the differentiation) actually caused a temporary, almost complete restoration of heart activity to the original indices. Here the action of the unconditioned stimulus had almost no effect on the altered operation of the heart. This indicates that, in such conditions, the influences on the heart which are realized through the cerebral cortex are expressed to a greater degree.

The question of whether collapse of the animal's higher nervous activity is unavoidable under the conditions described needs further study. We propose that, in the majority of animals, it is unavoidable in such experimental conditions. The reason for this seems to be the "petrifaction" of the previously developed stereotype.

The factual material obtained in the experiments described above shows the complexity of explaining the reasons for functional disturbances of heart activity. Actually, considerable disturbances in cardiac activity can be caused by factors which are seemingly usual and without effect on this activity, but which become "pathologic" stimuli when their signal meaning is changed, as well as by those factors which are known to change heart activity without fail. It is possible, therefore, that the environmental factors which have acquired a new signal meaning but which previously seemed to have no effect on the given activity could be regarded as usual and habitual and not considered in the search for the reasons underlying the functional disturbance of the body. They may escape attention, although a detailed study of changes in the meaning of the environmental factors usually affecting the body could help disclose a "pathologic" stimulus among them.

SUMMARY

Alterations of conditioned reflexes which we studied takes place in dogs after 3-4 combinations. In the neurosis which appears, the stimulus, which was formerly differential, enhances the disorder of the heart activity while the one that was formerly positive normalizes it.

Neurosis is maintained only for 14-17 days if the animals are removed from the room where experiments of alteration of reflexes were conducted. One 3-year-old dog completely recovered from neurosis in a fortnight.

The environment in which alteration of reflexes took place maintained disturbances of the heart activity for 5 months in a 9 year old dog, without administration of additional stimulation.

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• In Russian.

•• Original Russian pagination. See C.B. translation.

THE EFFECT OF PARTIAL EXTIRPATION OF THE PANCREAS ON THE DEVELOPMENT OF RECIPROCAL INHIBITION IN WARM-BLOODED ANIMALS

I. N. Volkova

From the Physiology Department of the Kazan Medical Institute (Director – Asst. Prof. R. A. Vyaselev)

(Received June 7, 1957. Presented by V. N. Chernigovsky, Active Member of the USSR Academy of Medical Sciences)

The studies of A. V. Kibyakov and A. A. Uzbekov [4] on cold-blooded animals and those of O. D. Kurmaev [6] on warm-blooded animals have shown that the synthesis of acetylcholine depends directly on the function of the pancreas. The removal of the greater part of the pancreas weakened the inhibitory effect of the vagus nerve on heart activity and caused a parallel decrease in the amount of mediator – acetylcholine – escaping into the perfusion fluid. The disturbance of the acetylcholine formation process occurring with partial extirpation of the pancreas was due to a disturbance of the phospholipid metabolism, since the works of many authors [8, 7] have proven that the pancreas plays an important role in the regulation of the fat-lipoid metabolism of the body. These data make it possible to employ partial depancreatization as a method of intervention in the acetylcholine formation process. This operation essentially has no effect on carbohydrate metabolism since, according to the data of Minkowski [9] and others, $^{9}/_{10}$ of the pancreas in warm-blooded animals can be removed without any great change in the blood sugar level. The fact that the disturbance of acetylcholine synthesis caused by partial depancreatization is only temporary seems to be due to the compensatory functional intensification of the remaining glandular tissue. All disturbances of parasympathetic system activity in depancreatized animals were almost completely eliminated when the animals were given a compensatory injection of pharmacological acetylcholine.

In our preceding investigations [1], we proved that partial extirpation of the pancreas causes sharp disturbances in the activity of the spinal centers in frogs. At specific post-operative intervals (from the 7th to the 10th day), we often completely failed to obtain reciprocal inhibition of the semitendinosus muscle reflex contraction, which was clearly defined in animals which had not had the operation. In connection with this, we decided to conduct analogous studies on warm-blooded animals, including additional research on the changes in the lability of the nerve centers with the normal development of reciprocal inhibition and after partial depancreatization, since we had already obtained data [2] demonstrating the importance of acetylcholine to changes in the lability of the spinal centers.

EXPERIMENTAL METHODS

The experiments were conducted on dogs under ether-chloroform anesthesia. The reflex tetanic contraction of the semitendinosus muscle in response to short (19-20 seconds), repeated (every 40-50 seconds) stimulation of the peroneal nerve was recorded by the current of an induction coil fed by an accumulator of 3 volts. In other experiments, we used a stimulator to stimulate this nerve, which permitted us to vary the stimulation frequency from 3 to 500 impulses per second without stopping stimulation. The limits of the afferent stimulation optimum and pessimum frequency were used as a cross index of the corresponding spinal centers' lability level. Reciprocal inhibition of the flexor reflex being recorded was obtained by contralateral stimulation of the same nerve for a period of 4-7 minutes. Part of the experiments were conducted on dogs in which the greater part of the pancreas had been previously removed under sterile conditions. The experiments were done at different

post-operative intervals, beginning with the 2nd and ending with the 20th-22nd post-operative day. As a control, we injected some of the depancreatized animals intravenously with a compensatory injection of 1-3 ml of pharmacological acetylcholine, in a dilution of 1 : 100,000. The injections were done daily, or 1-2 hours before the experiment. A total of 83 experiments were conducted.

EXPERIMENTAL RESULTS

In the animals which had not had the operation (11 experiments), stimulation of the peroneal nerve (distance between inductorium coils – 100-130 mm) caused a well-expressed reflex contraction of the semitendinosus muscle. With the addition of contralateral stimulation (distance between inductorium coils – 80-100 mm), the reflex being recorded was sometimes first intensified and then inhibited, but in the majority of experiments this additional stimulation caused the immediate lessening of the reflex, changing subsequently into profound inhibition (Fig. 1, a). We did not observe in any of the experiments with the animals which had not been operated upon the inversion of reflex inhibition in the form of the reestablishment of the originally inhibited reflex, in spite of continuing "inhibitory" stimulation.

Removing the greater part of the pancreas from the experimental dogs (30 experiments) caused essential changes in the character of spinal activity. The most obvious change was a comparatively rapid "exhaustibility" of the reflex reactions. Even ipsilateral stimulation caused the pessimal inhibition of the semitendinosus muscle reflex contractions. The reflex "exhaustibility" was greatest on the 6th-10th days after the operation. In these cases, we had to terminate the experiment without testing the effect of contralateral stimulation. In these same experiments, when the semitendinosus muscle reflex contractions in response to the short, repeated tetanizations of the peroneal nerve were still sufficiently well expressed, we added continuous stimulation of the same, but contralateral nerve to the stimulation of the ipsilateral nerve. We observed no evident disturbances in the normal development of reciprocal inhibition during the first days after the operation (3rd-4th). At later post-operative intervals (beginning with the 5th-6th and ending with the 11th-12th days), it was very difficult to obtain the development of reciprocal inhibition of the flexor reflex being recorded in the experimental dogs. Although we could still observe a weak reciprocal inhibition of the semitendinosus muscle reflex contractions on the 5th-6th day, on the 7th-8th-9th day the flexor reflex was usually not inhibited under the influence of contralateral nerve stimulation, in spite of the fact that the conditions of nerve stimulation remained the same (Fig. 1, b). In several experiments, we observed the inversion of the reciprocal inhibition, in which, in spite of the continuing "inhibitory" stimulation, the initially inhibited flexor reflex was unexpectedly reestablished (Fig. 1, c); this was never observed in the animals which had not had the operation. By the 11th-12th day, the disturbances in the activity of the spinal centers had begun to subside. On the 14th-15th day, the development of extremely profound reciprocal inhibition was observed in almost all of the experiments conducted. The inversion, and occasionally the impossibility of obtaining reciprocal inhibition in the depancreatized animals was also observed on a background indirectly recording the lability, for which the limits of optimal stimulation frequency in ipsilateral nerve stimulation served as the index.

The experiments made with animals which had not had the operation (12 experiments) showed that the addition of contralateral nerve stimulation caused various changes in the character of the semitendinosus muscle reflex contraction. On a background of "inhibitory" stimulation, the limits of the afferent stimulation optimum frequency in the flexor reflex showed a tendency towards slower rates in the majority of experiments, thus indicating a reduced lability in the corresponding center; transitional parabiotic stages became apparent – the equalizing and the paradoxical (Fig. 2, a). Sometimes, the decrease in lability which occurred with the development of reciprocal inhibition was preceded by some tendency of the optimal stimulation limits toward faster frequencies. After the inhibitory stimulation was stopped, the reflex being recorded was rather quickly reestablished, and the optimal stimulation frequencies returned to the original high levels.

In experiments with depancreatized animals (16 experiments), an average decrease in the optimal stimulation frequency to 17-12 impulses per second could be observed, and a decrease to 6 and 3 impulses per second in separate experiments, whereas the optimal stimulation frequency in the animals which had not had the operation consisted of an average of 33-50 and even 80 impulses per second. In the depancreatized animals, a tendency to more rapid pessimal inhibition of the reflex was observed under the influence of ipsilateral stimulation. However, along with the tendency to direct pessimal inhibition, sharp disturbances occurred in the development of the connected reciprocal inhibition. In the depancreatized animals, contralateral nerve stimulation usually did

not cause a change in the optimal frequency limits of ipsilateral stimulation towards slower rhythms as was observed in the normal animals, but, on the contrary, in a series of experiments caused the optimal stimulation limits to shift toward faster rates with no subsequent decrease (Fig. 2, b). An inverted reaction often appeared: the lability reduction in the flexor reflex center which began under the influence of contralateral stimulation was suddenly replaced by an increase in the lability – the limits of optimal stimulation frequency shifted to higher levels (Fig. 2, c). In several experiments, a decrease in lability was subsequently observed; however, this lability reduction was maintained or even further reduced when the "inhibitory" stimulation was excluded. This indicates that the inhibition of the flexor reflex is not caused by the development of reciprocal inhibition, but by the direct inhibitory effect of the ipsilateral stimulation itself. Such disturbances were greatest on the 6th-12th post-operative days.

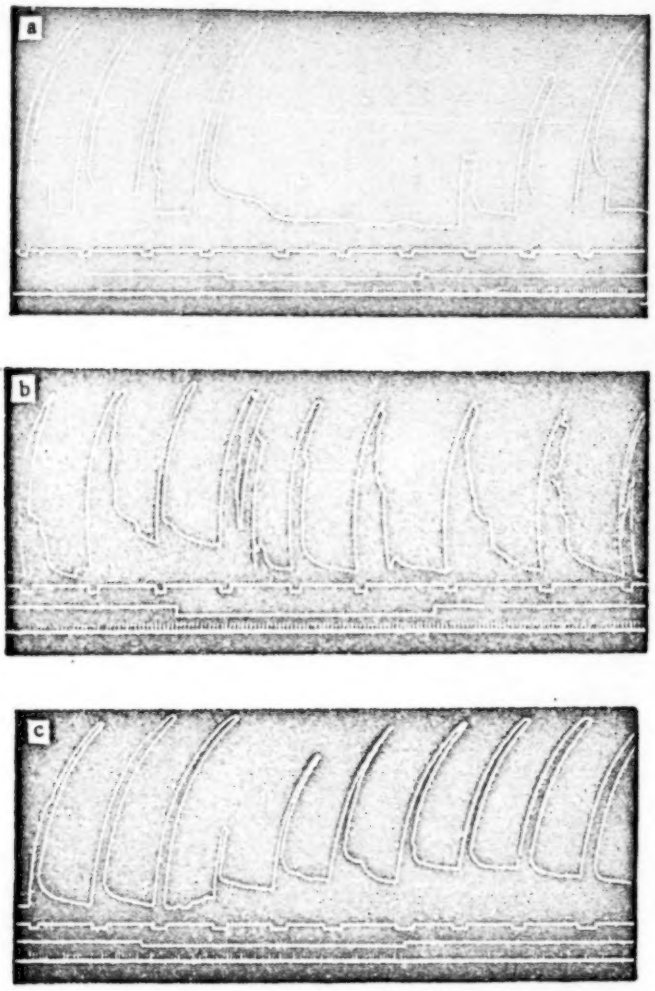

Fig. 1. The effect of partial depancreatization on the development of reciprocal inhibition in dogs.
a) development of reciprocal inhibition in dog which had not had the operation; b) absence of reciprocal inhibition on the 9th day after the operation removing part of the pancreas; c) inverted reaction on the 8th day after the operation. Curves from top to bottom signify: recording of semitendinosus muscle contraction; indication of peroneal nerve stimulation frequency; indication of contralateral stimulation; indication of time (in 5 second marks).

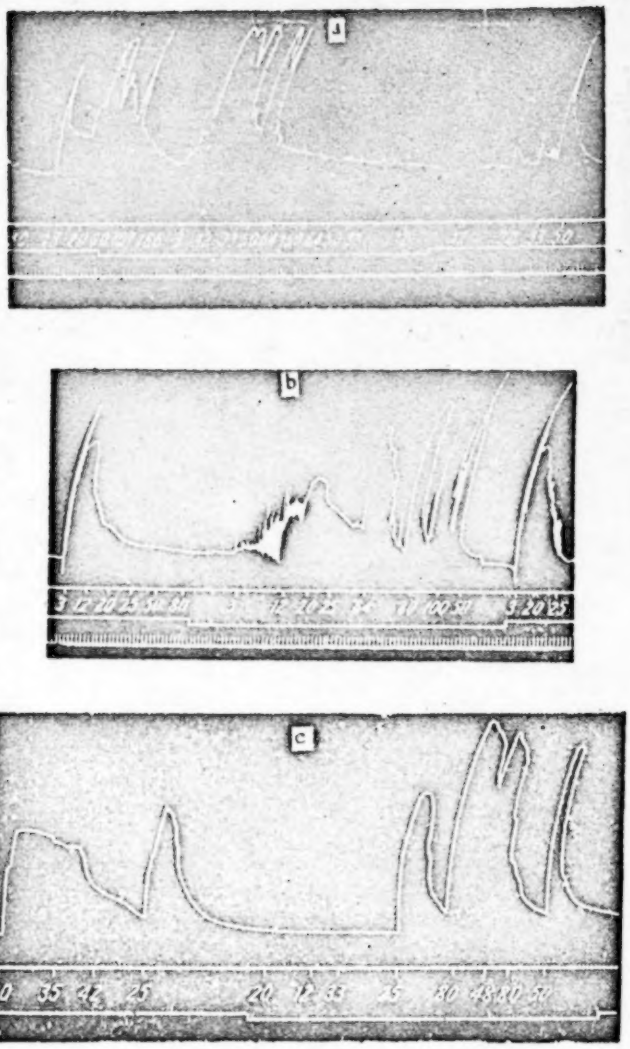

Fig. 2. Change of afferent stimulation optimal frequency on a background of contralateral nerve stimulation.
a) In dog which had not had the operation; b) on the 6th day after the operation partially removing the pancreas; c) on the 10th day after the operation. Curves mean the same as in Fig. 1.

In order to prove that the changes observed in spinal activity were specific, i. e. that they were due to the disturbance of the acetylcholine formation process, a group of the depancreatized dogs were injected with pharmacological acetylcholine (14 experiments). In the majority of cases in the control experiments, we succeeded in obtaining the development of reciprocal inhibition of the recorded reflex under the influence of contralateral stimulation (Fig. 3). This proved the specific character of the changes observed in the development of reciprocal inhibition in the depancreatized animals.

The changes we have described in the character of the reflex reactions of the animals operated upon are explained, we believe, by a change in the functional condition of the spinal centers, since the studies of L. N. Zefirov and A. V. Kibyakov [3] and of A. V. Kibyakov and N. E. Piontak [5] have shown that acetylcholine

synthesis disturbance has no noticeable effect on the development of the tetanic form of skeletal muscle contractive activity in the peripheral neuro-muscular apparatus.

Fig. 3. Development of reciprocal inhibition after compensatory introduction of acetylcholine (9th day after operation). Curves mean the same as Figs. 1 and 2.

Analysis of the results obtained permits the conclusion that acetylcholine synthesis disturbance in dogs causes essential disturbances in the development of reciprocal inhibition; in spite of continuing "inhibitory" stimulation, reciprocal inhibition either completely fails to develop or shows an altered reaction in the form of the reestablishment of the reflex. In a series of cases, the direct inhibitory influence of ipsilateral stimulation was added. The inverted development of reciprocal inhibition has been described by N. E. Vvedensky as occurring with extremely strong or prolonged stimulation of a sensory nerve. N. E. Vvedensky believes this phenomenon to be due to the development of inhibition in the center of the "antagonistic" reflex arc, which caused the reflex reaction being recorded opposite to be disinhibited. We also observed a similar inversion of the reaction in the animals which had had the operation in response to stimulation of moderate force and duration of the "inhibitory" nerve; this can only be associated with the reduced lability of the nerve centers and their tendency to more rapid transition to pessimal inhibition caused by the disturbed synthesis of a nervous activity mediator such as acetylcholine. These data, which prove the important role of acetylcholine in regulating the functional condition of the spinal centers, can be used to help explain a series of symptoms attending diseases of the pancreas.

SUMMARY

The disturbance of the synthesis of acetylcholine in the organism of warm-blooded animals by partial removal of the pancreas with simultaneous ligation of its ducts causes disturbance of the spinal activity. The total level of lability of the nerve centers is decreased, transmission to the pessimal inhibition is more rapid, but simultaneously the development of reciprocal inhibition is altered. It either does not occur at all with the usual conditions of stimulation or shows altered reaction in the form of reestablishment of the reflex, notwithstanding the continuing "inhibitory" stimulation.

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ON THE KINETICS OF THE CHOLINESTERASE OF FROG SMOOTH MUSCLE ORGANS AFTER REMOVAL OF THE PANCREAS

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As the numerous studies of A. V. Kibyakov and his colleagues have shown [3-8, 10], the removal of the pancreas in experimental conditions disturbs the influence of the parasympathetic nerves on the organs they innervate, changes the functional condition of somatic innervation in both its central and peripheral apparatuses and causes the disappearance of the so-called "spontaneous" contractions of the smooth muscle organs. These changes are connected with disturbances in the process of acetylcholine formation, since research by S. M. Leites [9] and others has shown that the pancreas plays an important role in the regulation of fat metabolism in the body, including phospholipid metabolism. The phospholipid metabolism is also closely connected with the process of acetylcholine synthesis. However, exactly how phospholipid metabolism disturbances affect the liberation of the mediator has not yet been completely investigated.

It seemed interesting to investigate the interrelations of the mediator, or acetylcholine, with the enzymatically-active systems and also the change of these interrelations when the phospholipid metabolism is disturbed by removal of the pancreas. In this article, we present experimental data from investigation of the fluctuations of cholinesterase activity in the smooth muscles of normal and depancreatized frogs.

From the literature data, we know that there are definite correlations between acetylcholine and cholinesterase. D. E. Alpern [1] shows that intensified acetylcholine synthesis is attended by an increase in cholinesterase splitting activity. The compensation condition is characterized by such a correlation. Along with this, humoral decompensation can also occur when the activity of cholinesterase is sharply reduced under the influence of strong stimulation of the vegetative nervous system parasympathetic branch and due to the liberation of a large amount of acetylcholine. N. F. Baranova and E. N. Speranskaya [2] also observed the decrease of cholinesterase with stimulation of parasympathetic nerve fibers. B. Mendel and H. Rudney [12] show that cholinesterase is inactivated by high concentrations of acetylcholine and gives maximal activity with low concentrations of acetylcholine.

EXPERIMENTAL METHODS AND RESULTS

Cholinesterase activity was determined by the titrimetric method described by T. V. Pravdich-Neminskaya [11]. According to this method, the acetic acid which forms, under the conditions defined by the method, from the enzymatic decomposition of acetylcholine is back-titrated by a 0.01 N solution of NaOH in the presence of an indicator – a 0.02% solution of cresol red. The percentage of acetylcholine destroyed in a unit of time is calculated, which is taken as the index of enzymatic activity. Titration was done with a solution of NaOH from a microburet with a total capacity of 0.6 ml with divisions every 0.002 ml. As the experimental objects in our experiments, we used: the muscular layer of the stomach, abdominal aorta tissue, and lung and bladder parenchyma. The femoral muscle of a frog was used for purposes of comparison. The tissues were ground in a mortar with a small amount of glass sand and centrifuged in a Ringer's solution at a rotation rate of 2000 revolutions. The centrifugate was used in the experiments.

In the first series of experiments, we determined cholinesterase activity in the smooth muscle organs and in the skeletal femoral muscle of normal frogs which had not been operated upon. These studies established that, during the fall-winter period, the cholinesterase activity in the smooth muscle organs of the frog ranged between: 3.00-6.28% for the stomach, 3.19-6.37% for the abdominal aorta, 3.00-5.82% for the bladder, 3.78-7.65% for the lung parenchyma. Cholinesterase activity increased sharply in the spring period, beginning with the second half of March, returning to the normal original levels in the middle of April. The maximal cholinesterase activity for the stomach muscles was 14.56%, for the abdominal aorta, 13.65%, for the lung parenchyma, 11.83% (Fig. 1) and for the bladder, 9.46%. The activity of the skeletal femoral muscle was stable (3.27-4.10%) in comparison with the smooth muscle organs and increased slightly towards the end of March (9.37%).

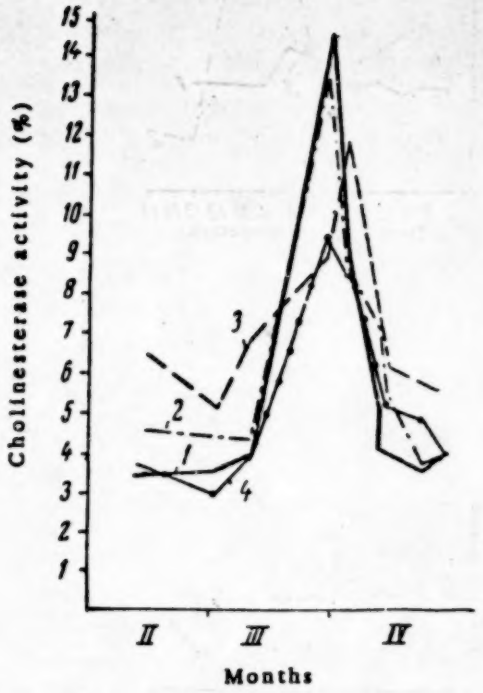

Fig. 1. Dynamics of changes in the cholinesterase activity of the smooth muscle organs of a frog at different months of the year.
1) muscular layer of the stomach; 2) abdominal aorta; 3) lung parenchyma; 4) bladder.

In a series of experiments, we injected 3-4 ml of eserine in a concentration of 1:10,000 into the subcutaneous posterior lymph sac of spring frogs (60 minutes before the experiment). The eserine injection caused a sharp decrease in cholinesterase activity, which ranged between the following limits: 2.21-5.73% for the stomach muscles, 2.28-6.37% for the abdominal aorta, 2.91-7.01% for the bladder, 3.00-7.19% for the lung parenchyma, and 3.55-6.55% for the skeletal muscle. Consequently, eserine preliminarily injected into the body of a spring frog lowered cholinesterase activity considerably; its magnitude under these conditions approximated the original, normal level, or was even somewhat less than the cholinesterase activity in the frogs of the fall-winter period.

The experiments described above make it possible to suggest the presence of seasonal fluctuations in the cholinesterase activity, which places it in direct dependence on the physiological phenomena which occur in the frog's body during the spring period after the winter hibernation.

In a second series of experiments, the pancreas was first removed from the frogs by operation. The experiments were done on frogs of the fall-winter period on the 2nd-14th day after the operation. It was established that cholinesterase activity in the depancreatized frogs gradually increased, beginning the second day after the operation. The cholinesterase activity of the stomach muscles and lung parenchyma was observed to increase until the 9th day after the operation, achieving a magnitude of 9.01-9.10%. Then the activity decreased and, after the 13th post-operative day, was reduced to the original normal range (Fig. 2, a, c). The change in the cholinesterase activity of the abdominal aorta was characterized by two sharp increases on the 3rd and 10th days after the operation (7.87% and 14.92%). After this, the activity decreased and only began to be reestablished after the 13th post-operative day (Fig. 2, b). A slower increase in cholinesterase activity was observed in the bladder tissues; by the 8th day after the operation, its magnitude equaled 6%, then the activity decreased and subsequently returned to the normal level (Fig. 2, d). A slight increase over normal cholinesterase activity (3.27-4.10%) was observed in the skeletal musculature (4.76%).

In order to prove that the changed cholinesterase activity in the organs of the depancreatized frogs was actually connected with the reduced amount of acetylcholine forming in the body, we conducted a third series of experiments in which depancreatized frogs were given a compensatory injection of pharmacological acetylcholine. The experiments, which were conducted on the days in which the greatest changes in cholinesterase

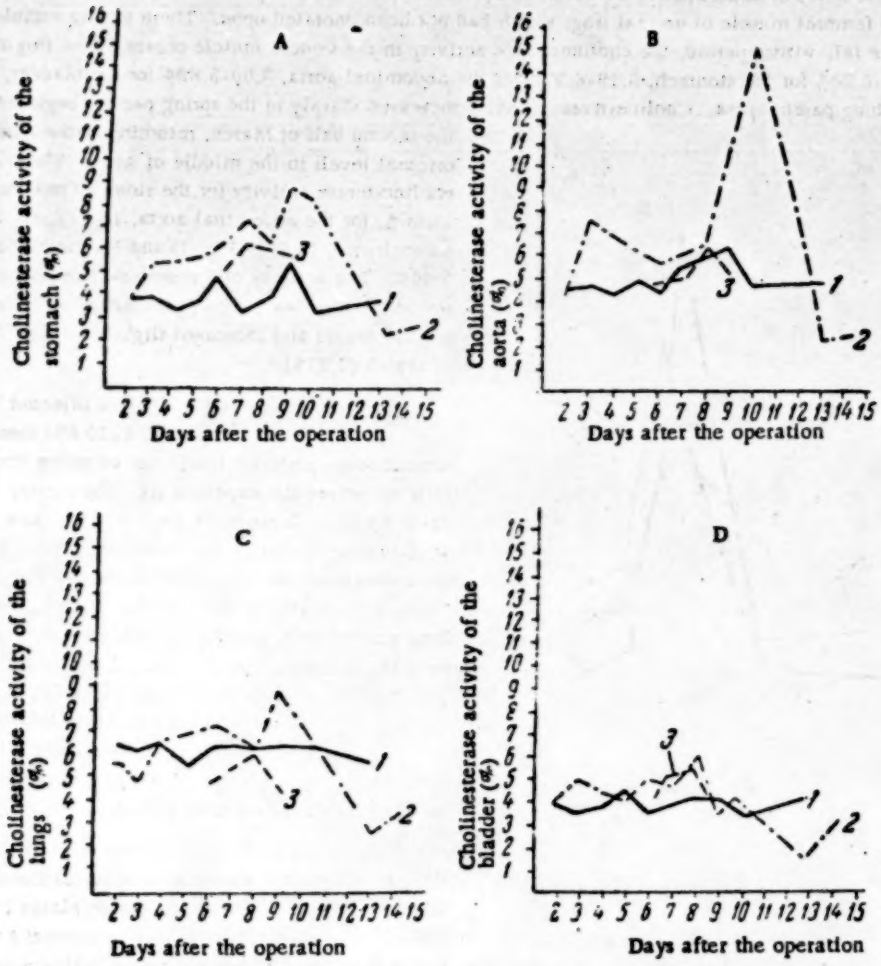

Fig. 2. Dynamics of changes in cholinesterase activity of smooth muscled organs of control frogs which had not been operated upon (1), of depancreatized frogs (2) and of depancreatized frogs which had been given a compensatory injection of acetylcholine (3).

content occurred (6th-9th days), showed that cholinesterase activity was almost normal or even slightly less than normal (see Fig. 2, a, b, c, d).

Our observations indicate that the removal of the pancreas in frogs causes considerable changes involving the increase of cholinesterase activity in the tissues. These changes in cholinesterase activity coincide in time with the disturbances of parasympathetic and somatic innervation which have been observed in many of our laboratory's works. From the experimental data obtained, we can conclude that, with the inadequate hormonal functioning of the pancreas, an increase is observed in cholinesterase activity which is probably the essential feature in the disturbed activity of the parasympathetic and somatic nervous systems which occurs in the body of depancreatized animals.

SUMMARY

Experimental data of the study of cholinesterase activity in the smooth muscles of normal and depancreatized frogs are presented. Variations in the activity of cholinesterase of smooth muscle organs of frogs are insignificant during the autumn-winter season. However, the activity of cholinesterase considerably increases during the spring season (March-April).

Increased activity of cholinesterase is, likewise, observed in depancreatized frogs. It coincides with the time of derangement of the parasympathetic influence on the organs and tissues during the postoperative period. Compensatory introduction of acetylcholine to operated frogs prevents the increase of cholinesterase activity.

It is probable that increased cholinesterase activity in tissues and organs of frogs is one of the factors of parasymphathetic disturbance in depancreatized frogs.

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ON THE 24-HOUR PERIODICITY OF CONDITIONED REFLEX ACTIVITY IN CHICKENS

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There is now sufficient data [3, 4, 6, 8] on the 24-hour periodicity of different physiological functions of both wild and domestic animals. However, information on the dynamics of nervous activity in animals over a 24-hour period is still scant.

In connection with the choice of a specific daily regime for the maintenance of farm animals, it is especially important to farm animal physiology to examine the features of nervous activity over a 24-hour period. It is now established that, when selecting a specific daily regime for domestic animals, one must take into account the physiologic properties of the specific breed as well as the agricultural-economic considerations.

Research on the daily periodicity of animal activity is especially interesting in connection with the possibility of controlling the basic nervous processes in order to regulate the periodicity of the internal functions of the body [5].

The method of actography and chronometry of the general behavior of animals over a 24-hour period, which has been widely used in research on the physiology of 24-hour periodicity and has given many valuable results, is not sufficient to completely disclose the dynamics and mechanism of the animal nervous processes in relation to the process of 24-hour periodicity. However, the conditioned reflex method of investigation makes it possible to obtain objective data evaluating the operation of the brain at different times during the 24-hour period.

In this work, we investigated the dynamics of nervous activity in chickens over a 24-hour period under natural maintenance conditions. As experimental subjects, we used Leghorn chickens which had been reared in laboratory conditions.

EXPERIMENTAL METHODS

The experiments were done on 8 chickens. A stereotype of conditioned reflex activity to color stimuli was developed in them by the motor-food method [1]. The stereotype consisted of two stimuli, positive and inhibitory, which were alternated in the experiment. We used a green monchromatic light filter (wave length—λ = 530 mμ) as the positive stimulus and a yellow as the inhibitory (λ = 585 mμ). The stimuli were used every 1 minute 30 seconds for 10 seconds, during which time they were used alone for 5 seconds and with a food reinforcement for the other 5 seconds. Food reinforcement lasted for 30 seconds.

The positive and negative associations developed rather quickly after 10-20 combinations. After the stereotype had been fixed, we examined the conditioned reflex activity of the chickens at various times during the 24-hour period.

Since the stereotype was developed on the basis of a food unconditioned reflex, in our investigations we had to calculate exactly the condition of food excitability. Therefore, when the stereotype was tested at

different times during the 24-hour period, we recorded the exact weight of the food given during the 24 hours and in the experiment. Moreover, in order to equalize food excitability, 60-80 g of food was given 1-2 hours before the experiment. The type of illumination was also taken into account; the animals were put in a light-proof chamber with artificial illumination 1-2 days before the experiment or on the day of the tests. No experiment was done more often than once a week. The background of conditioned reflex activity was always examined during the interval between the experiments.

EXPERIMENTAL RESULTS

The main point of interest was the difference between the morning and evening nervous activity of the chickens. Our basic test was to check the retention of the stereotype in the morning and in the evening (8-10 o'clock p.m.). One could expect that the magnitude of the positive conditioned reflexes would decrease in the evening hours due to the gradual exhaustion of the brain nerve cells of the chickens during the day.

The experiments conducted showed that a definite decrease in the magnitude of the positive conditioned reflexes actually did occur in the chickens at evening time. The magnitude of the positive conditioned reflexes was determined according to the length of the conditioned reflex latent period and was expressed in percent. The differentiation was determined in a similar fashion. The results of this study are given in Table 1.

TABLE 1

Change in the Magnitude of Positive Conditioned Reflexes in Chickens During the Morning and Evening Hours

Magnitude of positive conditioned reflexes, in %			
Morning (8-10 o'clock a.m.)	Number of tests	Evening (8-10 o'clock p.m.)	Number of tests
74.6 ± 1.8	73	50.7 ± 3.2	18

The differences discovered in the magnitude of the positive conditioned reflexes at different times of day are statistically true.

With the disappearance or reduced magnitude of the positive conditioned reflexes in the evening, a general inhibition and a state of hypnosis developed in the chickens during the experiment.

Having observed the reduction in the general magnitude of the positive conditioned reflexes which occurred during the evening, we found it interesting to trace the dynamics of conditioned reflex activity during the course of 24 hours. Therefore, the state of the conditioned reflex activity in the chickens was determined every 4 hours. To increase the strain on the animals' brains in order to determined their efficiency, the number of stimuli in the control testing was increased to 20. In order to show the 24-hour pattern of conditioned reflex activity, the chamber was illuminated around the clock.

From the experiments conducted in such conditions, we observed regular changes in the conditioned reflex activity of the chickens during the 24-hour period. The magnitude of the positive conditioned reflexes decreased towards evening, with the greatest reduction observed from about midnight to 4:00 a.m., and then increased again towards morning (Fig. 1). The differentiation remained about the same throughout the 24-hour period, becoming only slightly inhibited at 5:00 p.m. which was probably connected with a reduced concentration of internal inhibition. The results of this series of experiments agree with those obtained from research on the 24-hour pattern of conditioned reflex activity in monkeys [2, 7].

The motor activity in the chickens during the 24-hour period was recorded at the same time as the conditioned reflex activity. Here, we noticed that the changes in motor activity did not coincide with the changes in the magnitude of the positive conditioned reflexes in the course of the 24-hour period. Although motor activity in the chickens stopped at 6-7 o'clock p. m. with conditions of round-the-clock illumination (Fig. 2), the

maximal decrease in the magnitude of the conditioned reflexes was not observed until 1 o'clock a. m. During the experiments conducted in the evening, motor activity was observed to be restored in some chickens (see Fig. 2). The effect of the conditioned stimulus was so strong that the chickens, which had been asleep before its use, responded with a positive conditioned reaction. The question arose – what relations existed between the inhibition in the motor analysor and the state of stimulation which occurred with the action of the conditioned stimulus?. The greatest reduction of conditioned reflex activity, resulting in separate cases in the complete disappearance of the conditioned reflexes, occurred from midnight to 4:00 a.m. One must note that the greatest reduction of several physiological functions in animals possessing the day-time type of activity occurred during this time of the 24-hour period.

We know from I. P. Pavlov's theory that sleep is inhibition irradiated throughout the cerebral cortex. The phenomenon of successive inhibition occurs when internal inhibition is irradiated through the cerebral hemispheres. Similar relations were observed in our experiments.

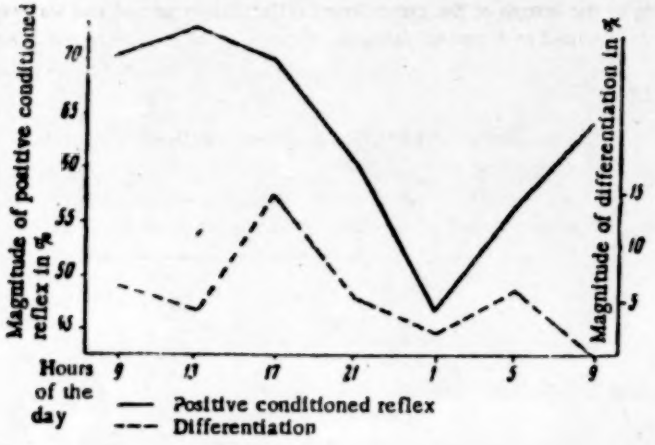

Fig. 1. Change in the magnitude of positive and negative conditioned reflexes in chickens during the course of a 24-hour period.

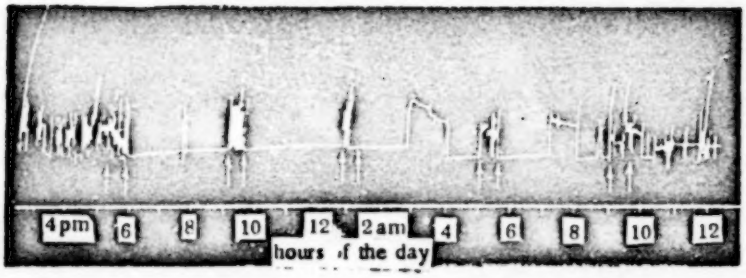

Fig. 2. Recorded motor activity of a chicken during a 24-hour period in conditions of round-the-clock illumination. The arrows show the times at which the experiments were conducted.

The phenomenon of successive inhibition was often recorded in the evening experiments; this phenomenon occurred after the use of differentiation stimuli and was expressed by either a considerable prolongation of the reflex latent period or by the complete disappearance of the reflexes. Usually this occurred at the end of the experiment.

In order to specifically trace the development of successive inhibition, we changed the stereotype so that all the differentiation stimuli were used first and then all the positive stimuli were used. This method of conducting the morning and evening experiments showed that the greatest development of successive inhibition was in the evening; part of the chickens lost some of the reflexes completely and others went to sleep. The results of the experiment are shown in Table 2.

TABLE 2

Magnitude of Positive Conditioned Reflexes After the Use of 4 Differentiation Stimuli at the Beginning of the Stereotype

Number of chickens in experiments	Magnitude of positive conditioned reflexes, %	
	Morning (8-10 o'clock a.m.)	Evening (8-10 o'clock p. m.)
8	66.50	42.00

From the results of this experiment, one can affirm that the greatest irradiation of internal inhibition occurs in the evening, and therefore the development of successive inhibition.

We also studied the features of extinguishing inhibition in order to ascertain the properties of higher nervous activity in the chickens during the evening. The process of extinguishing the positive conditioned reflex was rather complex; it could characterize the force and mobility as well as the steadiness of the nervous processes. Nevertheless, it was interesting to examine the development rate of extinguishing inhibition during the morning and evening hours.

The reflex was extinguished at 30 second intervals until 5 and 10 negative reactions to the positive stimulus were obtained. The experiments showed that the development rate of extinguishing inhibition was slower in the evening, especially when the extinguishing process was continued until 10 negative reactions were obtained. Similar data were obtained in experiments on monkeys [7].

The results of the experiments permit us to make the following conclusions.

The properties of higher nervous activity in chickens change regularly in the course of a 24-hour period. The efficiency of the brain nerve cells declines in the evening. In conditions of round-the-clock illumination, we observed 24-hour pattern of conditioned reflex activity, expressed by a reduction in the magnitude of the positive conditioned reflexes during the evening and night and by an increase of these indices in the morning hours. The greatest reduction in the magnitude of conditioned reflex activity occurs from midnight to 4:00 a.m.

There is a greater development of successive inhibition in chickens during the evening than in the morning.

Extinguishing inhibition develops more slowly in the evening than in the morning.

The conditioned reflex method of research is one of the most important ways of studying 24-hour periodicity in animals.

SUMMARY

The 24-hour rhythm of the conditioned reflex activity was studied in Leghorns. The value of conditioned reflexes was determined every 4 hours to the positive and negative (alternating during the experiment) color monochromatic stimulants. The motor food method was used. The deepness of the successive inhibition and the rapidity of formation of extinguishing inhibition during the morning and evening hours was determined in other series of experiments. The presence of 24-hour rhythm of conditioned reflex activity of chickens was established. The most pronounced decrease in the conditioned reflex activity of chickens occurs from midnight to 4 o'clock in the morning. The successive inhibition is enhanced in the evening, while the development of the extinguishing inhibition is delayed.

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THE ROLE OF THE NERVOUS SYSTEM IN LACTATION

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The presence of nerve trunks servicing the mammary gland has been established by many researches; these nerves have been shown to originate from the lumbar-sacral section of the spinal cord, and their branches in the gland have been traced [3, 4, 6, 11]. Similar studies by P. Dmitrievsky [5], later confirmed by other works, proved that a mass of effector type nerve endings are present in the glandular cells of the mammary gland. Research conducted by M. Mironova (1895) and L. N. Voskresensky (1895) in I.P. Pavlov's laboratory showed first that lactation depends on the integrity of the nerve trunks and secondly that lactation is changed by stimulation of these nerve trunks. The latest studies of I. I. Grachev [3] showed the importance of the interoceptive reflexes to mammary gland activity.

However, there are certain known experimental data from which scientists have concluded that humoral factors, particularly the hypophysial hormones, play the only regulating role in lactation. The supporters of the humoral theory of lactation are W. Petersen, F. Goltz, O. Riddle and others. They discount the role of the nervous system in the lactation process.

EXPERIMENTAL METHODS

In this work, we investigated the effect on lactation of stimulating and inhibiting the autonomic nervous system by means of pharmacological substances.

The pharmacological method makes it possible to avoid the negative influence of operative intervention, which may damage nerves and vessels, and provides conditions for observation which are maximally proximate to normal conditions. Changes in the tonicity of the autonomic system may be estimated according to the operation of glands known to be regulated by the central nervous system, for example, the salivary glands, and by then comparing these data with the effect on the mammary gland. The substances we used for this purpose were:

1. Pilocarpine and carbocholine (Merck's "Lentin"), as substances stimulating the parasympathetic part of the autonomic nervous system, especially its secretory fibers. We used the following doses: 1.5-2 ml of 1% pilocarpine and 0.4-1.5 ml of a 0.1% solution of carbocholine.

2. Atropine, as an agent inhibiting the nervous elements, in a dose of 1-1.5 ml of a 1% solution.

3. Adrenalin, as a stimulator of the sympathetic part of the autonomic nervous system. It was injected in doses of 1.5-2 ml of a 0.1% solution.

4. Thyroidin, which was fed with bread in a daily dose of 2 grams. The sympathicotropic action of thyroidin was the principal effect we had in mind, and we therefore used thyroidin in several experiments in combination with adrenalin in order to intensify thyroidin's effect on the sympathetic division.

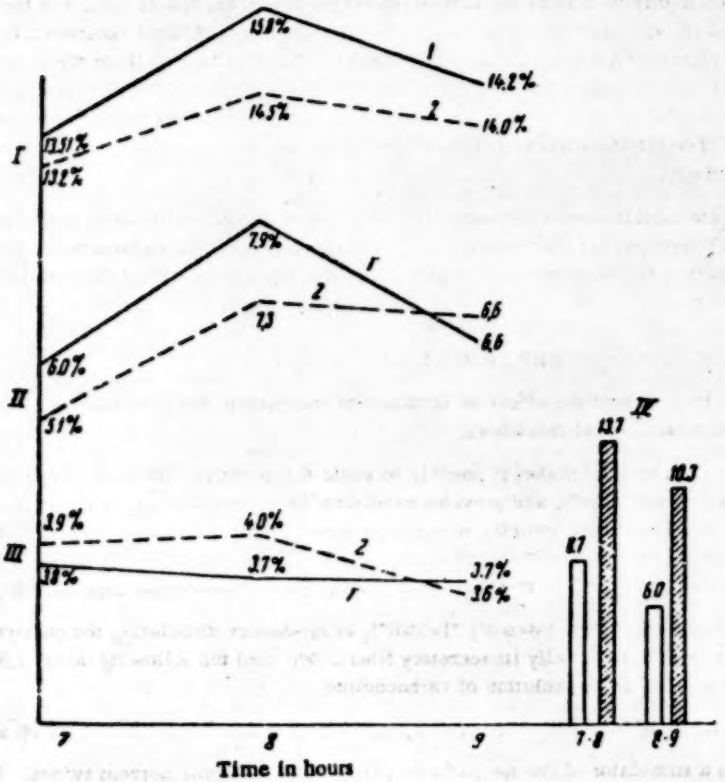
The experiments were conducted on 7 animals – 5 female goats and 2 cows. After a control period, the substance was injected daily for 4-7 days; a week elapsed between each series of experiments. The injections of the experimental substance were done in the morning. The udder was completely stripped to eliminate all

accumulated milk before the injection. The subsequent batches of milk were obtained by careful milking 1 and 2 hours after the injection. The changes in the composition of the milk were also studied, which made it possible to estimate the effect of the experimental preparation on the corresponding portion of the nervous sytem and also to ascertain the character of the secretion. For this purpose, we studied the changes in the dry matter content and in certain of its components – fat, lactose and ash.

To observe the effects of the substances and the change in the tonicity of the autonomic nervous system, fistulas of the parotid gland ducts were induced in 3 goats. This enabled us to calculate the intensity of saliva secretion.

EXPERIMENTAL RESULTS

1. The effect of the substances stimulating the parasympathetic part of the autonomic nervous system – pilocarpine and carbocholine – on the lactation process was expressed by an increase in the volume secreted the first hour after the injection. With pilocarpine, this increase was an average of 15% in relation to the control experiments for the first hour after the injection, and reached 56% in individual cases. With the carbocholine injection, there was a greater increase in the milk yield, which consisted of an average of 145% in relation to the control experiments the first hour after the injection, and in individual cases was more than 200%.



The effect of carbocholine on change in milk composition and on the intensity of the synthesizing work of the gland (average from 5 goats).
I) dry matter; II) percentage of fat in milk; III) lactose; IV) yield of dry matter in g per hour; 1) carbocholine; 2) control; shaded column – carbocholine; white column – control.

The effect of carbocholine was more lasting; it was still clearly evident in the second hour when the milk yield had increased by 93% in separate animals, with an average increase of 64% in relation to the control experiments. An increase in the dry matter content of the milk was also characteristic of the effect of these poisons, especially of carbocholine; when we computed the absolute dry matter content of the whole milk from the hour's yield, we found that the injection of these preparations had increased the gland's production of dry matter both absolutely and relatively, i. e. had stimulated the glandular activity. Thus, with the carbocholine injection, the amount of dry matter in the milk obtained after an hour consisted of 13.7 g, as compared with 8.7 g in the control experiments.

Therefore, the parasympathicotropic substances used also affected the secretory activity of the gland. This was also indicated by some change in the fat content of the milk. Although the usual dynamics of fat content changes (increase in fat content with repeated milking) were maintained, in separate animals during the experimental period the carbocholine injection caused the amount of fat in the milk to increase by 0.7-2%; the fat content was sometimes as high as 10%. On the average of all the experimental goats, however, no substantial increase in the fat content of the milk was observed during the first 2 hours, although the morning milk was fatter by 0.9% during the period of the carbocholine injection. Consequently, in this respect the effect of carbocholine was rather lasting. The sugar and ash content did not change; the protein content (see Figure) possibly increased.

Carbocholine also caused saliva secretion to be almost doubled. Thus, the normal saliva secretion for a 3-minute period being 3-5 drops of saliva; 10 drops were secreted during the same 3-minute period 24 minutes after the injection. These data confirm the effectiveness of the poison dose, establish the increased tonicity of the parasympathetic section of the nervous system as a whole and also show that this substance affects both glands in the same way.

	In the control experiments	After the atropine injection	% in relation to the control exp.
Average morning milk yield	490	447	91.3
Milk yield for the first hour	59.8	32	53.5
Milk yield for the second hour	43	23	54.2
Daily yield	1147.6	1095	95.5

From our observations of salivary gland operation, we could establish the very important fact of the reflex interaction between the mammary and salivary glands. We found that either milking or udder massage alone caused saliva secretion to increase from 1-2 drops to 3-7 drops in a 3-minute period. The carbocholine injection, which raised the tonicity of the parasympathetic elements of the nervous system, sharply intensified this reflex reaction of the salivary glands, so much so that, during milking, the saliva was secreted in a continuous stream.

	In the control experiments	After the atropine injection
Average milk yield from the 5 goats	5.1	4.3
Average milk yield for the first hour	7.3	6.9
Average milk yield for the second hour	6.7	5.9

2. Atropine had an effect opposite from and more strongly and clearly expressed than that of carbocholine and pilocarpine. Atropinization of the animals caused a sharp reduction of lactation both the 1st and 2nd hours; the amount of lactation was 47-85% in relation to the control experiments.

Atropine's effect on the size of the milk yield (in millimeters) is shown in the averages taken from the five goats.

The inhibitory effect of atropine on secretion lasted a long time, causing the day's milk yield to decrease by 20-30%. The morning milk yield on the day after the drug injection was also reduced.

The fat content of the milk was characterized by the following data (in percent).

The decreased percentage of fat in the milk of the morning yield was evidently the result of the action of the atropine on the preceding day.

Atropine's effect on the salivary gland was typical and reduced not only the "spontaneous" saliva secretion, but also the reflex saliva secretion effected by milking. Before the atropine injection, the goats secreted 4-5 drops of saliva every 3 minutes. Fifteen minutes after the atropine injection, no saliva drops were secreted, and 1-2 drops of saliva appeared during milking, although there was no milk. An hour after the atropine injection, milking produced 15 ml of milk, and 2-3 drops of saliva were secreted in 3 minutes; there was no saliva 2 hours after the atropine injection, but 5-7 drops of saliva were secreted during a 3-minute period during milking, which produced 30 g of milk.

Therefore, there is a similarity between the activities of the mammary and salivary glands, which indicates the proximity of their regulator mechanisms.

3. The effect of the sympathicotropic substances was manifested as follows. The adrenalin injection caused the secretion volume to increase considerably both for the first 2 hours and for the whole day: there was an increase of 86% in the yield of the first hour and of 26-36% in the 24-hour milk yield, although the effect of adrenalin is usually short-lived and the adrenalin itself quickly disintegrates. It is possible that the nervous system stimulation caused by the adrenalin lasted considerably longer than the adrenalin itself. Thus, in the experiments on goats, adrenalin affected the volume of milk (in milliliters) as follows.

	In the control experiments	After the adrenalin injection	% in relation to the control experiments
Average morning yield	490	528	107.7
Yield for the first hour	59.8	111.2	186
Yield for the second hour	43	59.8	139
24-hour milk yield	1147	1446	126

Still more characteristic was the considerable increase in the dry matter content of the milk, which is shown by the following data (in percent).

	In the control experiments	After the adrenalin injection
In the morning yield	13.18	13.20
In the yield for the first hour	14.5	16.3
In the yield for the second hour	14.0	15.27

Of the dry matter components studied, only the amount of fat and, to a lesser degree, the amount of ash changed; the sugar content remained the same. Since the increase in the dry matter seemed more than the increase of the fat, sugar and ash, one can propose that the protein content also increased rather considerably. Therefore, the sympathicotropic substances obviously affect the intensity of the synthesizing processes in the gland, i. e., milk secretion.

Increase of Dry Matter Content of the Milk in Grams

	In the control experiments	after the adrenalin injection	% in relation to control experiments
" second "	8.67	18.15	209
" second hour	6.02	9.13	151

The thyroidin feeding caused an increase in both the hourly and daily milk yield. This indicates that thyroidin, which raises the metabolism of the body, at the same time intensifies the excitability of the nervous system. The use of thyroidin combined with adrenalin led to the further increase of secretion volume or to stimulation caused by this combination of substances if they had had no effect individually.

Indices	Control experiments	After injection of		
		thyroidin	adrenalin	thyroidin + + adrenalin
Percentage of dry matter (average) in milk of morning yield	14.1	16.6	18.4	14.2
Average yield for a day (in ml)	1860	2264	1851	2020
Morning yield, ml	753.3	980	810	777
Yield for first hour, ml	186	175	143	200
Yield for second hour, ml	73	95	51	130
24-hour yield, in % of control experiments	100	122	99	109
Yield for first hour in % of control experiments	100	94.1	76.8	103.2
Loss of dry matter, g:				
a) in the first hour milk yield	32.3	31.0	28.0	30.0
b) in the second hour milk yield	10.6	16.7	10.8	19.8
c) total, without "a" and "b"	219.4	324.7	238.6	224.8
Total in 24 hours, g	262.2	372.3	277.5	273.9
Total in 24 hours (in % of control experiments)	100	142	106	104

The table gives data concerning the changes in the amount of milk yield and in the dry matter content of the goat milk after the injection of the sympathicotropic substances. Evidently, the metabolism intensification caused by thyroidin is conducive to the manifestation of the effect of the adrenalin.

The experiments with atropine and carbocholine were repeated on the two cows, with results similar to those obtained in the experiments on the goats.

In the first place, the material obtained amends data concerned with the effect of autonomotropic substances on the lactation process which are quoted in certain abstracts (in D. Espe [8], for example). In the second place, the material obtained shows that, in principle, the autonomotropic substances have the same effect on the mammary gland as on other glands in which nervous regulation of secretion is irrefutably established. This similarity of effect evidently indicates that the regulator mechanisms directing the activity of these glands are similar, and leads to the conclusion that not only the udder blood-supplying and milk discharge processes are regulated by the central nervous system, but also the actual secretion of milk.

The sharp changes in lactation caused by atropine refute the opinion expressed by American scientists that the hypophysial hormones play the leading role in the secretion and discharge of milk. These authors established that atropine cannot neutralize the effect of oxytocin and pitressin (V. Petersen [9]). An experiment blocking the

central nervous system by alcohol anesthesia was conducted on 2 goats. Milk was not secreted for 3 hours. As the effect of the anesthesia began to wear off, the mammary gland began to function.

This study confirms the possibility of direct nervous system influence on mammary gland activity and also the reflex effect of mammary gland stimulation on other organs, which facts help to strengthen and develop I. P. Pavlov's theories of neurism in a region where they have heretofore been criticized.

SUMMARY

The influence of changes in the condition of the nervous system due to the effect of autonomic agents (pilocarpine, carbocholine, atropine and adrenalin) and thyroidin on the secretion and content of milk in goats and cows was investigated. The above research was carried out together with the study of the secretion of salivary glands, as well as of the tone of the autonomic part of the central nervous system. This gave the opportunity for better understanding of the relationship between the organism as a whole and the mammary gland. It was established by this work that the introduction of pilocarpine and carbocholine into the organism, as well as of the sympathicotropic substances – adrenalin and thyroidin – causes increased secretion and the change of content of milk. Increased quantity of dry residue in milk is the result of increased synthetic activity of the cells of the mammary gland under the influence of excitation of the nervous system by the above substances. Block of the parasymphathetic nerve endings by atropine causes pronounced decrease of milk secretion. However, it is not completely arrested. The process of milking and massage of the udder causes excitation of the central nervous system resulting in reflex increase of salivary secretion. The mammary gland receives impulses which appear due to stimulating effect of milking, sucking and massage. Nervous and nervous humoral mechanisms take part in it under the control of the central nervous system.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

EXPERIMENTAL MYOCARDIAL INFARCT IN CERTAIN PHYSIOLOGICAL AND PATHOLOGICAL STATES OF THE ORGANISM

PART 3. EFFECT OF HYPERTENSION IN THE PULMONARY CIRCULATION ON THE DEVELOPMENT OF EXPERIMENTALLY INDUCED MYOCARDIAL INFARCTION

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In our preceding two communications [4, 5] we examined the effect of graded physical exertion and of hypertension in the systemic circulation on the development of myocardial infarction. Our experiments showed that physical exertion and hemodynamic changes due to raised arterial pressure had a considerable effect on the origination and development of myocardial infarction.

The development of experimental myocardial infarction was affected to a particularly large extent by raised arterial pressure, with resulting overloading of the left ventricle, and this determined the dimensions of the lesion, its extension, and its slower healing.

In connection with these findings, we thought it would be of interest to study the effect of overloading of the right ventricle on the development of myocardial infarction, in particular on its localization and extension.

It is known that functional overloading of the right heart is encountered in a number of diseases of the lungs (emphysema, bronchial asthma, pneumosclerosis), in certain diseases of the cardiovascular system (mitral stenosis, lesions of the minor branches of the pulmonary arteries, thromboses, and pulmonary artery embolisms), and in other conditions, such as kyphoscoliosis. The heart-lung syndrome arising in these conditions represents a complex of hemodynamic disturbances with hypoxemia and functional overloading of the right heart (V. F. Zelenin [7], B. B. Kogan [8, 9], T. Durant [13], P. Soulie [15], M. Gelfand [14]). Some authors (O. Brenner [12], H. Spencer [16]) also distinguish the condition of primary hypertension in the pulmonary circulation.

These hypertensive conditions of the pulmonary circulation, hypoxia, and overloading of the right ventricle, can undoubtedly be associated with local disturbances of venous flow and with myocardial infarction. It may be supposed that the development of myocardial infarction will be particularly affected by acutely developing functional overloading of the right heart, as in bronchial asthma, pulmonary artery thromboses and embolisms, and bilateral generalized pneumonias. In such cases we encounter raised pressure in the pulmonary arterial system, together with raised venous pressure, imperfect filling of the ventricle (so-called "small heart"), lowering of the minute volume, retardation of the blood flow, increase in the volume of circulating blood, fall in systemic blood pressure, lowered basal metabolism, and hypoxemia. Within the venous system we find retardation of blood flow, together with venous stasis and obstructed lymph flow.

It is known from clinical observation that similar complexes of functional overloading of the right heart and of generalized circulatory disturbances with localized disturbances of the coronary circulation can exert a most unfavorable effect on the course and prognosis of myocardial infarction; this question has not, however, received full consideration in the published literature. It is generally believed that lesions of the right ventricle are of very rare occurrence, because of the large number of anastomoses between branches of the left and the

right coronary arteries, and of the role of the vessels of the Thebesian system, which can to some extent compensate deficient blood supply of the thin-walled right ventricle. The only literature references which we could find were to the prevalence of right sided heart lesions in emphysema (S. S. Vail [1, 2]), fat embolism of the lungs (S. A. Vinogradov [3], N. T. Raikhlin [11]), influenzal pneumonia (F. L. Leites [10]), cardio-pulmonary insufficiency (O. A. Zakharova [6]), croupous pneumonia, tuberculosis, pneumonias in children, and other conditions.

The object of the present research was to ascertain to what extent hypertension in the pulmonary circulation and overloading of the righ ventricle can affect the site, extension, and healing of a myocardial infarct, caused experimentally by ligating the anterior descending coronary artery.

EXPERIMENTAL METHODS

The experiments were performed on rabbits, of which we used 42 in all. The results were compared with those found for a second group of 36 animals, in which the coronary artery was ligated, but no other measures were taken.

In the basic series of experiments, functional overloading of the right ventricle was achieved by constricting the pulmonary artery (by means of a silk ligature, inserted under the artery) by about a third of its normal diameter, or by inducing fat embolism of the lungs. Localized disturbances of coronary circulation were produced, as in the control group, by ligation of the descending branch of the anterior coronary artery, at the margin of the left auricle, and below the point of emergence of the septal branch. Myocardial infarction was produced in 31 animals (ligation was ineffective in 4 rabbits, and the remaining 7 animals died after the first or second stage of the operation).

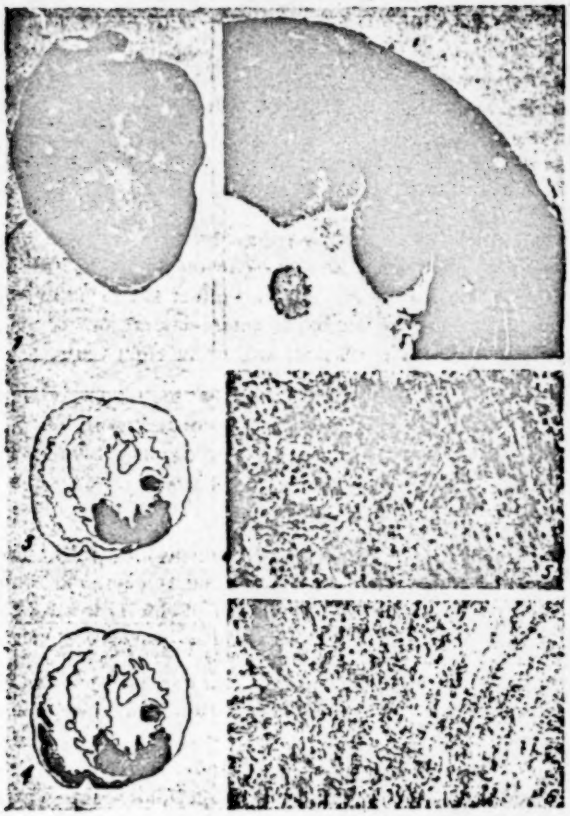
Number of Rabbits Used in Experiments on Production of Myocardial Infarction

Duration of infarction	Functional overloading of the right ventricle, caused by				
	constricting the pulmonary artery			fat embolism of the lungs	
	simultaneously	before ligation	after ligation	before ligation	after ligation
To 6 hours	—	1	—	2	—
12 »	1	—	—	1	—
24 hours	1	—	—	—	—
2 days	3	—	—	1	—
3 »	2	—	—	3	2
4 »	2	—	—	1	—
5 days	2	—	1	—	—
6 »	1	1	—	—	—
8 »	1	—	—	—	—
10 »	2	—	—	—	—
20 »	1	—	—	—	—

As a control, ligatures were placed around the pulmonary arteries of 2 animals with myocardial infarction, but were not tightened. The diameter of the lumen of the pulmonary artery of the remaining animals was reduced by about one-third. Fat embolism of the lungs was produced by injection of 1 ml of castor oil into an ear vein.

Electrocardiographic recordings were taken throughout the period of observation. Apart from the standard leads, we applied 3-5 thoracic leads (1-2 leads to the right and the left of the apex beat). After the death of the animals we filled the coronary vessels with contrast medium, and studied them by means of stereoangioroentgenograms [5]. The heart was examined in histotopographic sections, cut from the whole organ embedded in

celloidin-paraffin blocks, and stained with hematoxylin-eosin, picrofuchsin, fuchselin, or toluidine blue, or silver-impregnated according to Gomori.



Changes in the myocardium of rabbits subjected to experimental infarction.
1) Experimental aneurysm of the right ventricle; 2) propagation of the infarct to the anterior and lateral wall of the right ventricle (duration of observation 10 days; photographed through a lens); 3) diagram showing location of an infarct caused by ligating the descending branch of the anterior coronary artery (control experiment); 4) diagram showing location and extension of an infarct under conditions of functional overloading of the right ventricle; 5) part of an infarct of the anterior wall of the left ventricle, on the 4th day after ligation of the descending branch of the anterior coronary artery; a proliferative-cellular reaction in necrotic foci, with a preponderance of histiocytes and round cells (stained hematoxylin-eosin, magnification obj. 10x, oc. 10x); 6) lateral wall of the right ventricle (from the same preparation); fresh necrosis and leucocytary infiltration-progression of the infarct, with extension to the wall of the right ventricle (fat embolism of branches of the pulmonary artery, 24 hours before killing the animal).

EXPERIMENTAL RESULTS

The stereoroentgenograms of the heart vessels of control animals showed that, as in the human, a considerable part of the anterior wall of the right ventricle is supplied by branches of the anterior descending coronary artery. Notwithstanding this, we found, in a control series of experiments, that ligation of the anterior descending branch of the left coronary artery, with resulting infarction of the anterior wall of the left ventricle and of the anterior part of the interventricular septum, did not involve the right ventricle, apart from insignificant infiltrations affecting parts immediately adjacent to the septum (see Figure, 3). Necroses of the anterior wall of the right ventricle were observed in the control group only when it had been injured by the needle, or at the level of the ligature.

Under the conditions of the experiment itself, i. e., when localized disturbances of the myocardial circulation were combined with functional overloading of the right ventricle, we found that infarction regularly extended to the anterior wall of the right ventricle (see Figure, 4). This effect was found in 24 of 29 animals, in 8 of which the lesion extended not only to the anterior, but also to the antero-lateral wall of the right ventricle. In a number of cases we also observed infarction of parts of the lateral wall of the right ventricle (8 animals; see Figure, 2).

A detailed analysis of our experimental material showed that the dimensions of the infarct and of its central necrosis in the left side of the heart, in animals with pulmonary hypertension, did not differ significantly from what was found in the control group of animals, subjected to coronary ligation alone, other than in extension of the infarct to the walls of the right ventricle. Only in 5 of this series of animals did the dimensions of the infarct and of its central necrotic focus exceed those found in the control series.

The degree of leucocyte infiltration, the preponderance of juvenile cells, and the speed of healing depended on the size of the necrotic focus. This persisted for about 10 days, and could not be seen in specimens taken 20 days after ligation. In 4 animals the process had extended chiefly towards the wall of the right ventricle, with formation of fresh foci of more recent circulatory disturbances, of necroses, and of infiltration.

In 2 or 3 animals, in which pulmonary circulation had been impeded, and right heart overloading achieved some days after ligation of the anterior descending coronary artery with formation of a left ventricular infarct, the lesions observed in the walls of the right ventricle were of more recent origin than were those of the left ventricle and the septum. Thus when a pulmonary embolism had been produced 2 days after origination of an infarct in the wall of the left ventricle, we found a proliferative-cellular reaction at the site of the infarct, with large numbers of histiocytes and round cells (see Figure, 5), whereas the wall of the right ventricle contained more recent necroses, with massive leucocyte infiltration (see Figure, 6); the animal was killed 24 hours after injection of oil.

The above observations are evidence that under conditions of raised pressure in the pulmonary circulation, with functional overloading of the right ventricle, progression of infarction can take place, with formation of fresh foci of necrosis and infiltration in the myocardium, and with extension of the infarct to the walls of the right ventricle. The extent of the right ventricular lesions was in some cases so great as to lead to attenuation of the walls, with formation of aneurysm-like bulges (see Figure, 1).

Examination of the histotopographic sections revealed foci of necrosis and cellular infiltration, among which were vessels containing contrast medium; this is evidence that these vessels were not completely shut off from the circulation. At later stages, a pronounced sclerotic process was evident in the regions affected by necrosis and infiltration, the scar tissue extending in places across the whole thickness of the wall of the right ventricle; in some places not even the subendocardial layer survived, although it usually remains intact in myocardial infarction, irrespective of its localization.

In control experiments, in which we constricted the pulmonary artery without ligating the coronary artery, we observed dystrophic changes in the wall of the right ventricle, a mucoid edema of the stroma, minute areas of proliferation and of necrosis, and subsequent mild sclerotic changes in the wall of the right ventricle.

The electrocardiographic data obtained from animals suffering from myocardial infarction complicated by functional overloading of the right heart also testify to the extent of the lesions of the anterior walls of the heart, and in general give a picture corresponding with infarctions of the extent decribed above. A more detailed analysis of these data requires a special description•.

• The electrocardiographic studies were conducted jointly with E. A. Kyandzhuntseva in the Electrocardiographic Laboratory of V. N. Vinogradov, Member AMN SSSR.

Our experimental findings on the role of functional overloading of the right ventricle in the development of myocardial infarction afford, to a certain extent, an explanation of the gravity of myocardial infarction under conditions of raised pressure in the pulmonary system, such as is present in bronchial asthma, lung embolism, and other conditions.

SUMMARY

When the anterior descending coronary artery is ligated and there is a simultaneous narrowing of the pulmonary artery or the fat emboli of the lungs the infarction usually spreads to the anterior or, in certain cases, to the antero-lateral and the lateral wall of the right ventricle in rabbits. In certain cases there were noted, likewise, the formation of the aneurysm of the right ventricle with advance of the process and appearance of fresh foci of necrosis and infiltration in the wall of the right ventricle. Thus, in the functional overstraining of the myocardium there is incoordination between the circulation of the blood and increased requirement of nutrition of the heart muscle. This brings about advancement of the process of myocardial infarction with affection of the functionally overloaded areas of the myocardium.

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EFFECT OF EXPERIMENTALLY INDUCED HEART DISEASE ON CONDITIONED REFLEXES INHIBITING RESPIRATION AND THE ACTIVITY OF THE HEART

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The view that the nervous system plays an important part in the mechanism of the compensatory process developing in heart disease was first advanced by S. P. Botkin [2], on the basis of his extensive clinical material, and has been studied experimentally by Timofeev [7], working with I. P. Pavlov [6], Ballint [8], Hulmangyi et al. [10], L. Ya. Balonov [1], and others.

Insufficient attention has, however, been paid to the actual mechanisms whereby continuous compensatory hyperfunction of the heart, and the timely deployment of extracardiac compensatory factors, are assured in heart disease.

We have shown in our previous communications [4, 5] that in the stage of stabilized compensation of heart defects experimentally induced in rabbits there was a pronounced weakening of the unconditioned reflex reaction to stimulation of the upper respiratory passages with ammonia vapor, and consisting of respiratory arrest and bradycardia. The duration of the unconditioned reflex apnea and the degree of bradycardia are 3-5 times smaller in rabbits with heart defects than in controls. This change is associated with strengthening of sympathetic nerve reflexes augmenting the activity of the heart. Thus a specific readjustment of nervous centers takes place, ensuring uninterruptible hyperfunctioning of the defective heart, and excluding the possibility of reflex inhibition of respiration and cardiac activity.

The present paper describes the results of a study of the effects of cardiac defects on conditioned reflexes formed on the basis of unconditioned reaction to exposure of the upper respiratory passages to ammonia.

EXPERIMENTAL METHODS

The animal material consisted of 16 male rabbits, weighing 2-2.5 kg.

Experimental aortic stenosis was produced by the method described in a previous paper. The aortic cross section was reduced to a quarter to one sixth by the application of a silk ligature. Stenosis was associated with the characteristic x-ray and morphological changes in the heart. In the first group of experiments, on 6 rabbits, we formed a stereotype of conditioned reflexes, and aortic stenosis was then induced in 4 of them; a ligature was applied to the aorta of the remaining 2, but was not tightened. The conditioned reflexes of all 6 animals were then followed during the 10 days after the operation, i. e., up to the stage of stable compensation of the defect, and after the effects of operational trauma and of the initial severe reactions to the stenosis had passed off. In the second group of experiments we formed conditioned reflexes in 4 rabbits which had been subjected to experimental aortic stenosis a month earlier, and in 6 control rabbits, which had not been operated on at all, with the object of ascertaining the effect of a pre-existing heart defect on the process of formation of conditioned reflexes.

All the experiments were conducted with the animals in the prone position. Respiration was recorded on an electrokymograph, and cardiac activity by means of an electrocardiograph (EKP-4M), with a fitted electrical recorder of stimulations.

Unconditioned reflex apnea and bradycardia were caused by irritating the upper respiratory passages with ammonia. In the unoperated rabbits the duration of reflex apnea was 15-20 seconds, and bradycardia was manifested by an abrupt fall in the heart rate from 250 to 30-50 beats per minute, returning to normal within 20-30 seconds.

The conditioned reflexes were formed on the basis of the given unconditioned reflex. The conditioning stimuli were a metronome, frequency 300 beats per minute, and a green light. The differentiating stimulus was a metronome of frequency 30 beats per minute. The duration of exposure to the conditioning stimuli was 10 seconds. Delaying was not at first practiced; the conditioned stimulus was applied immediately after exposure to ammonia. A delay of 1 second was then instituted, and this delay was gradually increased, up to 6 seconds. The conditioned reflexes appeared after 4-8 combinations, and a constant delay of 6 seconds was reached after 15-20 combinations.

Until the reflex was formed neither M-300 nor green light had any perceptible effect on the respiratory and ECG tracings. After its formation the isolated sounding of M-300 caused apnea lasting as long as it was sounded, with pronounced bradycardia, involving retardation of heat rate from 240-260 to 60-80. The light stimulus caused equally pronounced reactions. Differentiation (M-30) was formed after 6-10 associations, and was incomplete in about a third of the animals, i. e., some retardation of respiration and heart rate was observed. The conditioned reflexes were of great stability, and could only be extinguished after 25-30 unreinforced stimulations.

The respiratory and cardiac components of the conditioned reflexes were of parallel intensity, and for convenience we usually assessed the strength of the reflexes from the respiratory component only. The strength of the reflexes was calculated by determining a mean normal value for respirations per minute for each animal, and then finding the number during a period of 6 seconds.

If, for example, the respiratory rate was 75 per minute, then there would be $7\frac{1}{2}$ respirations in 6 seconds. We then counted the number of breaths shown on the pneumograms during a 6 second period of action of the conditioned stimulus. If the number of breaths was the same as before stimulation, e.g., $7\frac{1}{2}$, it followed that the conditioned stimulus had no effect on the respiratory rhythm, and the strength of the conditioned reflex was 0. If, on the other hand, respiration was arrested with the moment of application of the conditioned stimulus, and was absent during the whole of the 6-second period of stimulation, the strength of the reflex was at a maximum, and amounted to 100%.

If the respiratory rate was half the normal value during stimulation, the strength of the reflex was 50%.

The strength of the conditioned reflex was found to vary from 60 to 100% during a given experiment, in a group of 6 control rabbits.

The strength of the reactions to the auditory and visual stimuli bore a constant relationship. The magnitude of the reflex to M-300 was 70-100%, and of the reflex to green light 27-50%.

EXPERIMENTAL RESULTS

The previously described weakening of unconditioned reflexes was observed 10 days after constriction of the aorta of all 4 rabbits of the first group, in which conditioned reflexes had been formed before the operation; the duration of unconditioned reflex apnea was reduced from 15-20 to $7\frac{1}{2}$-$12\frac{1}{2}$ seconds, and the degree of bradycardia from 30-50 to 100-120 beats per minute. The strength of the conditioned reflexes fell correspondingly. The strength of the auditory conditioned reflexes to M-300 fell from 100-60 to 40-27%, and of the conditioned reflex to light to 0. The corresponding conditioned reflex bradycardia fell from 30-50 beats per minute to 120-200 beats per minute, and was altogether absent in some experiments (Figs. 1 and 2). There was no change in the reaction to the discriminating stimulus. The extent of weakening of conditioned reflexes varied for different animals, parallel to the weakening of unconditioned reflexes in the given rabbit.

Application of a ligature to the aorta, without causing stenosis, caused a certain retardation of heart rate, and led to prolongation of unconditioned reflex apnea. No significant changes were observed in the strength of conditioned reflexes in these two animals.

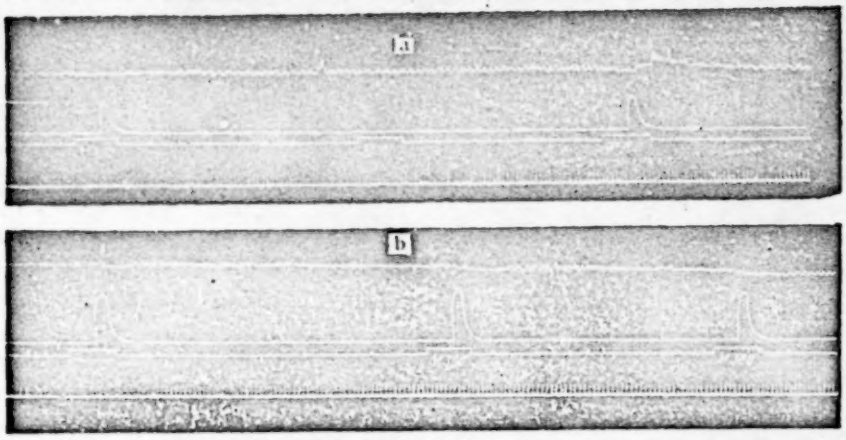

Fig. 1. Conditioned reflexes in a normal rabbit (a), and in the same animal a month after constriction of the aorta (b).
Explanation of curves (from above down): respiration, unconditioned stimulus, conditioned stimulus, time marker.

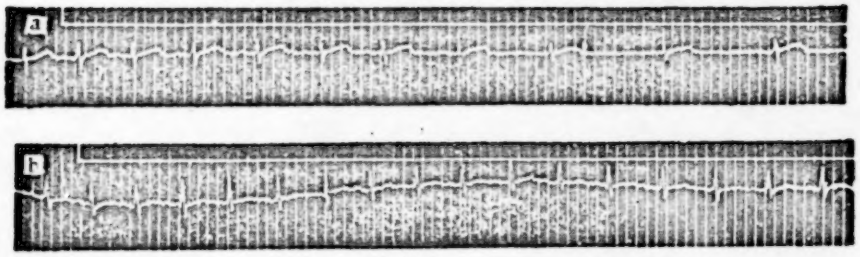

Fig. 2. Conditioned reflex bradycardia in a normal rabbit (a); in a rabbit which had been subjected to aortic stenosis a month earlier (b) – bradycardia is absent.
The upper tracing shows time and duration of the auditory stimulus.

In the second group of experiments the formation of conditioned reflexes in response to the M-300 auditory stimulus in animals with pre-existing aortic stenosis was, naturally, more difficult, in view of the weakening of the unconditioned reflex. The first appearance of the conditioned reflex was observed after 3-7 associations, in both groups of animals, with or without aortic stenosis. The two groups diverged widely thereafter, however. Whereas the strength of the conditioned reflex varied in the control group from 60 to 100% after 15 associations, it rarely exceeded 35-40% in the group with aortic stenosis, even after 35 associations. Apart from this, the operated group differed from the control in that stabilization of the reflexes did not occur – failure to respond occurred periodically.

The weakness and the instability of the conditioned reflexes in rabbits suffering from heart defects was a hindrance to formation of delayed reflexes. Whereas a delay of 6 seconds was achieved in the control group after 13-15 associations, this was not possible in the operated group even after 35 associations.

SUMMARY

A reduction of the unconditioned reflex, which caused apnea and bradycardia was noted in animals with compensated experimental aortic stenosis. The sound and light conditioned reflexes which were formed on its base were, likewise, reduced. The process of formation of the new conditioned reflexes on the base of the above unconditioned reflex became more difficult. These experimental data were evaluated in view of I. P. Pavlov's conception of the closure of the conditioned association between the cortical representation of unconditioned reflex and the cortical representation of the corresponding conditioned stimulant. It may be suggested that the reduction of the above unconditioned reflex in heart defect causes decreased excitability of its cortical representation. As a result of this the power of the process of excitation is decreased, the existing temporary associations are disturbed to a certain degree and the formation of new temporary associations becomes more difficult. The adaptive significance of reduction of unconditioned and conditioned reflexes, which inhibit the heart activity consists (in condition of heart defect) in the fact that it more or less guarantees the vitally important continuity of the compensatory hyperfunction of the heart.

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DISTURBANCES IN AND RESTORATION OF FUNCTIONS OF VISCERA AFTER CLINICAL DEATH

PRELIMINARY COMMUNICATION. DISTURBANCE AND RESTORATION OF GASTRIC FUNCTION AFTER CLINICAL DEATH OF 2 AND 7 MINUTES DURATION

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Most of the studies made of the changes taking place in the organism of higher animals dying of acute hemorrhage, and subsequently resuscitated, have been related to biochemical and electrophysiological changes in cerebral and myocardial activity, and to basal metabolism [1, 2, 6, 7], as well as to certain aspects of higher nervous activity [3, 4, 5]. Up till now, no work has been done on the study of the changes which take place, as a result of fatally terminating blood loss, in the functioning of a number of the internal systems of the body, and in the first place in the alimentary tract and the kidneys.

We had at our disposal a dog called Malchik, 5-6 years old, a cross between an Alsatian sheepdog and a farmyard dog. This animal had been in the laboratory for a number of years, and was accustomed to staying immobile in a harness for long hours. It had been subjected to gastrotomy and esophagotomy in 1952, and had adapted well to feeding through a stomach tube inserted through an opening at the lower end of the ligated esophagus. The dog had been fed twice daily for 4 years, i. e., to the end of May 1956, during which time its weight had remained steady at about 17-18 kg. The following were examined: 1) the secretory function of the stomach, in response to 3-minute sham feeding with raw meat; 2) total acidity of the gastric juice; 3) the digestive power of the gastric juice, assessed according to Mett; 4) nature of the fasting (periodic) activity of the stomach, registered by V. N. Boldyrev's method.

EXPERIMENTAL METHODS

The experiments were started after thorough washing out of the stomach with warm water, for which purpose we used not less than 3 liters of water, after which we recorded the amount of juice secreted over a period of from 30 to 60 minutes; the dog was then sham-fed for 3 minutes. The latent period of gastric secretion was recorded, and the amount of juice secreted per 15 minute period was measured over 1-2 hours.

Peak secretion was usually observed during the first hour of the experiment. For this reason we frequently restricted our observations to one hour after sham feeding. The gastric juice collected during this hour was filtered, and its total acidity was determined by titration with N/10 NaOH, using 1% alcoholic phenolphthalein as indicator. Digestive power was assessed from the number of millimeters of protein column digested after 12 hours of incubation at 37.5°. Periodic stomach contractions were registered through air transmission on an electrokymograph, with the drum rotating at a rate of 50 cm in 15 minutes.

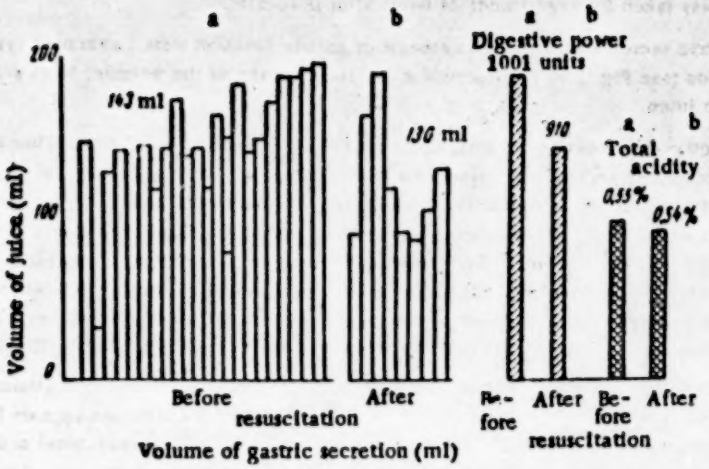

Fig. 1. Amount of gastric secretion (1st experiment).

EXPERIMENTAL RESULTS

The dog was twice bled to death. During the preceding 3 months we performed 22 experiments involving sham feeding with subsequent determination of volume, total acidity, and digestive power of gastric juice secreted during the next hour (Fig. 1, a). The dog was first bled to death on May 31, 1956 (Protocol No. 1979). Its weight was then 17.5 kg. Before the experiment we gave the dog a 2% solution of Pantopon, at a dosage level of 2 ml per 5 kg body weight. The femoral vessels were exposed under local procaine anesthesia. The volume of blood withdrawn amounted to 960 ml.

The death agony was of short duration, lasting for 2 minutes 46 seconds. Clinical death lasted for 2 minutes. Cardiac activity returned 30 seconds after commencement of intra-arterial transfusion of blood and of artificial respiration. The first spontaneous inspiration appeared $1^1/_2$ minutes after commencement of resuscitation. Spinal reflexes reappeared 3 minutes 45 seconds after resuscitation began, corneal reflexes in the left eye after 6 minutes 15 seconds, and in the right eye after 7 minutes, while tendon reflexes reappeared after 7 minutes

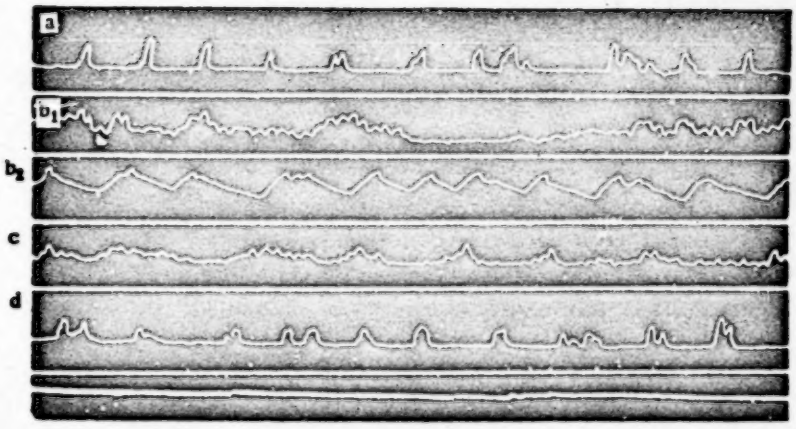

Fig. 2. Characteristics of "hunger" contractions.
a) experiment of April 24, 1956 (control); b_1) experiment of June 3, 1956 (3 days after resuscitation); b_2) continuation of the experiment of June 3, 1956; c) experiment of December 11, 1956 (4th day after second resuscitation); d) experiment of February 14, 1957 (2 months after second resuscitation); below: time signal (1 sec.).

The animal reacted to light and sound after 11 hours. It began to walk on the following day, with a very uncoordinated gait. It was taken for experiment 44 hours after resuscitation.

Studies of the gastric secretion and of other aspects of gastric function were executed systematically over a month. Over this period (see Fig. 1 b) we observed a certain lowering of the volume, total acidity, and digestive power of the gastric juice.

Periodic gastric activity was examined 9 times in control experiments, and 7 times after resuscitation. Judging from the duration (summary) of the periods of activity before and after bleeding, no great change in the nature of this activity had taken place. Before the experiment, the mean duration of activity periods was 28 minutes, and of the pauses 43 minutes. After resuscitation the average length of the activity periods was increased to 33 minutes, while the length of the pauses remained unchanged. There were, however, considerable changes in the form of the contractions, which were well-marked and of considerable amplitude in the control experiments (Fig. 2, a), whereas for a long time after resuscitation they had a chaotic character, and consisted of slow, feeble waves of low amplitude (see Fig. 2, b_1 and b_2). Only after a lapse of 3 months was the normal periodic activity of the fasting stomach restored.

After a 2-month interval (July-September) the observations were resumed at the beginning of September, and were concluded on the 7th December, when the dog was again bled to death. As appears from Fig. 3, a, the volume of gastric secretion varied within relatively narrow limits over this period; total acidity and digestive power fell slightly, but remained at about the same levels (0.49% HCl and 757 enzyme units) over the 3-month period of observation. The periodic activity of the stomach gradually reverted to the normal state. On December 7, 1956 the dog was again bled to death, the intention this time being to prolong the duration of clinical death to 5 minutes.

The experiment was conducted similarly to the first one; (Protocol No. 2112; the vessels were exposed under local anesthesia, and general anesthesia was not applied). The agonal period lasted for 2 minutes 30 seconds. The volume of blood withdrawn amounted to 1150 ml. Regular breathing ceased 3 minutes 30 seconds after bleeding was begun, and was followed by a terminal pause, lasting for 22 seconds. This was succeeded by an agonal period lasting for 3 minutes, during which time corneal reflexes disappeared. All reflexes had disappeared 5 minutes after bleeding was begun. Resuscitative measures were instituted precisely 5 minutes after clinical death had been established. Cardiac activity recommenced 7 minutes 25 seconds later. The delay in re-establishment of cardiac activity was due to the appearance of ventricular fibrillation at the beginning of the resuscitation period; this was abolished by means of single condenser discharges. The heart resumed normal activity 50 seconds later. Thus the total duration of the state of clinical death was 7 minutes 30 seconds.

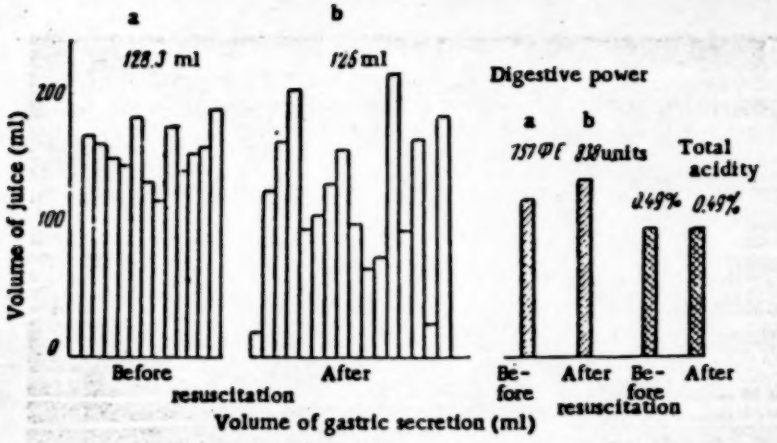

Fig. 3. Gastric secretion (2nd experiment).

The first spontaneous inspiration was observed 8 minutes 30 seconds after resuscitation was begun. In spite of repeated administration of adrenalin vascular tonus was not recovered until after intravenous injection of 0.1 ml of ephedrine solution at the 13th minute of resuscitation. Pupillary reflexes were present at the beginning of

the 15th minute, spinal reflexes 14 minutes 20 seconds after commencement of resuscitation, corneal reflexes after 15 minutes 35 seconds, tendon reflexes after 16 minutes 40 seconds, and palpebral reflexes after 17 minutes 5 seconds. After 2 hours the dog was deeply unconscious, and the extremities were in extension, and somewhat rigid. Milk containing 0.15 g of Barbamyl* was introduced through a stomach tube. The dog remained curled up on its side for 20 hours; it reacted to light and sound, and responded to the voice of the experimenter by raising its head and licking its lips.

After 42 hours it tried to jump out of its bed, and greedily approached food. It was taken for the usual sham feeding experiment after 3 days.

During the subsequent 2 months the secretory activity of the stomach showed wide fluctuations in the amount of juice produced, from 18 ml during the first hour of an experiment to 216 in another. The acidity also showed wide variations, from 0.39 to 0.56%, and the digestive power from 3 to 9 mm. The volume of gastric juice secreted during 1 hour (mean of 15 experiments) was 126 ml, which is only slightly less than was found during the preceding 2 months, viz., 128.3 ml.

Much the same applies to the mean values for total acidity and digestive power of the juice (see Fig. 3,b). Considerable changes were evident in the periodic activity of the stomach, chiefly due to an even greater prolongation of the periods of activity, whereas the length of the pauses between these periods remained much the same as after the first bleeding. The average increase in the length of the periods of activity was 10 minutes (44 instead of 34 minutes).

Our experiments thus show that both the secretory and the motor functions of the stomach are affected to a greater or smaller extent after different durations of clinical death due to exsanguination. It appears from our preliminary data that the most considerable and the most persistent changes are in the periodic activity of the stomach (so-called "hunger" contractions), which had not fully reverted to normal even after 2-3 months following exsanguination. The secretory function is affected to a smaller degree, although wide fluctuations in the hydrochloric acid content, digestive power, and volume of gastric juice secreted are seen.

Work is being continued on this dog, and on a number of other animals similarly treated.

Our further experiments will show the basic cause of the changes observed by us in our experiments.

SUMMARY

The author has studied the secretory and motor ("hunger" periodical motions) stomach activity in the gastro-oesophagotomized dog. Following the establishment of standards of the activity under investigation, the dog was twice bled to death in short experiments. The clinical death lasted 2 minutes in the first experiment and 7 minutes in the second. Then the changes of the stomach activity and the dynamics of the processes of recovery were studied. Exhaustion of the secretory function of the organism and chaotic motor function of the stomach were revealed. The process of recovery took from 2 to 4 months.

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INDUCTION OF HYPOTHERMIA BY DIRECT COOLING OF THE BRAIN THROUGH ITS EXTERNAL COVERINGS

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The changes observed in the organism as a result of its general cooling are in the first place due to progressive inhibition of the central nervous system. The result of this inhibition is to produce step-wise abolition of its functions, beginning with the cerebral cortex. Conditioned reflex activity is abolished in dogs when the body temperature falls to 28-30° [4]. Changes take place in the electrical activity of the cerebral cortex, which becomes weaker as the temperature falls, finally ceasing altogether [10, 12, 3].

Disturbance of function of the between-brain and the mid-brain takes place much later [10], while the functions of the medulla oblongata and the spinal cord persist to lower temperatures, and are the last to be abolished [1, 4].

Procurement of profound inhibition of the central nervous system by means of generalized lowering of body temperature is now widely applied clinically, in particular in connection with heart surgery [12, 8, and others]. The induction of generalized hypothermia, especially in its more extreme forms, is by no means a safe or a simple undertaking, either in the human or in animals. One of its most dangerous complications in surgical practice is the development of ventricular fibrillation, in particular when the heart has been excluded from the circulation. The frequency of incidence of fibrillation is in direct proportion to the degree of cooling of the organism [8, 9, 15]. This fibrillation greatly limits the possibilities of applying hypothermia. It is of importance to find ways and means of preventing its onset.

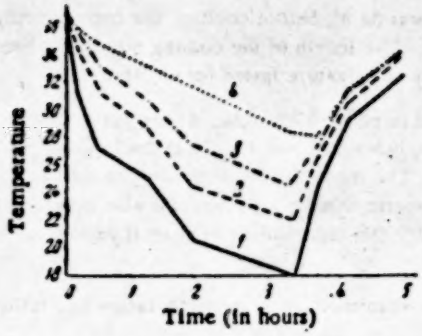
The useful effect of hypothermia under conditions of exclusion of the action of the heart is that it protects the central nervous system, and in particular the cerebral cortex, from the harmful effect of the resulting anoxia, since the tolerance of the brain to oxygen lack is much higher in the hypothermic state [6, 2]. It follows from this that the most important aspect of hypothermia is the lowering of the temperature of the brain, and in particular of its higher levels. This points to the possibility of inducing hypothermia by cooling the head alone, leaving the rest of the body at a relatively high temperature; since this includes the heart, it may fully prevent its fibrillation. The cooling of the brain through the cranium is quite feasible, since of all tissues bone is the best conductor of heat. It should in this connexion be mentioned that Nikitinov [7] proposed the application of cold to the head for the treatment of certain brain conditions, as far back as 1885.

Attempts at clinical applications of cold to the head have been described in the literature. Thus Fay and Smith [13], who treated cancerous disease by hyperthermia, applied ice-packs not only to various parts of the body of the patient, but also to the head. Fay [14], in the treatment of headaches of varied etiology, used a special helmet placed on the head, with a stream of cold water running through it.

The present paper describes a study of the induction of hypothermia by cooling the brain alone, through its external coverings, and of the temperature profiles achieved at different levels.

EXPERIMENTAL METHODS

In all, we performed 17 experiments on cats. Cooling was effected by means of a small cooling blanket, in which the head of the animal was wrapped. The hair had first been thoroughly removed from the head. Water at 3-5° was circulated through the blanket. Temperature readings were recorded simultaneously from two or three levels of the brain, using a copper-constantan thermocouple, the insulated function of which was enclosed in a hollow needle with a closed end. At the same time rectal temperature was measured by means of a mercury thermometer inserted to a depth of 8 cm.



Rate of fall and rise of temperature at different levels of the brain and in the rectum during external cooling of the head, with subsequent warming of the organism. 1) Upper layer; 2) middle layer; 3) deep layer; 4) rectum. Cat, body weight 2.8 kg. Experiment of the 4th May 1956.

In some of the experiments we did not insert the thermocouple needles, in order to avoid injury to the brain, but judged the brain temperature from that of the blood returning from the brain in the jugular vein. For this purpose we inserted a very thin and smooth thermocouple into the vein, against the direction of the current of blood. Control experiments showed that the temperature of the blood was very close to that of the brain.

Arterial pressure was recorded from the carotid artery by means of a mercury manometer, and respiration by means of a Marey capsule, connected through a four-way tube to the trachea. Ether anesthesia was applied.

Restoration of brain temperature was achieved by warming the whole body with an electric heater until the rectal temperature reached 33-34°, with a short period of heating of the head at the beginning of the recovery period.

EXPERIMENTAL RESULTS

Different temperatures were recorded from different levels of the brain (upper, middle, deep) during the cooling process. As is evident from the Figure, the temperature fell fastest in the outer layer, and more slowly in the middle one (subcortical region). The temperatures of the basilar regions of the brain differed considerably from the two preceding regions. The temperature of the rest of the body fell to some extent, but remained high, relative to the brain. At the end of the period of cooling, the brain temperatures (means of 10 determinations) were: upper layer (cortex) about 18°, middle layer (subcortical) 21.2°, while the rectal temperature was 29°. The temperature of the base of the brain (mean of 4 experiments) was 24.7°.

TABLE 1

Changes in Arterial Pressure, Heart Rate, and Respiratory Rate During External Cooling of the Brain (temperatures relate to the mid-brain). Means of 9 Experiments

Function \ Brain temperature	Cooling phase					Rewarming phase		
	37°	35°	30°	25°	20°	25°	30°	33°
Arterial pressure, mm Hg	137	140	114	101	93	106	122	134
Heart rate, beats per minute	176	172	160	134	110	114	130	158
Respirations per minute	27	24	23	19	14	19	18	23

Thus the characteristic feature of our experiments was the considerable temperature difference between the brain, and particularly of its upper layers, and the rest of the body.

The same regularity was evident when jugular vein temperatures were measured; rectal temperature remained at a high level, as compared with jugular vein temperature. Thus when the rectal temperature was 36.5° (mean of 8 measurements), and the jugular vein temperature was 35.8°, before cooling, the corresponding temperatures at the end of the cooling process were 30.9° and 21.8°. The length of the cooling process for these experiments was 90 minutes, on the average. Return to normal body temperature lasted for 94 minutes.

Cooling of the head alone was associated with circulatory and respiratory changes. As the temperature fell in the brain, there was a gradual fall in arterial pressure, which, however, rose slightly at the beginning of the cooling process, when the brain temperature was 35° (Table 1). The mean fall in pressure when mid-brain temperature was 20° was to 67.8% of the initial value (mean of 9 experiments). The heart rate also fell gradually, to 62.5% of the initial value when mid-brain temperature was 20°. No disturbances of heart rhythm were observed during the cooling process.

The respiratory rate had fallen to about half the initial value when the mid-brain temperature had fallen to 20°, amounting on the average to 51.8%.

Similar effects were found in the experiments in which jugular vein temperatures were measured (Table 2). Slight shivering was noted during the cooling process.

TABLE 2

Changes in Arterial Pressure, Heart Rate, and Respiratory Rate During Cooling of the Brain (temperatures measured in the jugular vein). Means of 8 Experiments

Jugular vein temperature / Function	Cooling phase					Rewarming phase		
	36°	34°	30°	25°	22°	30°	32.5°	35°
Arterial pressure, mm Hg	145	138	120	104	86	110	121	129
Heart rate, beats per minute	202	182	168	138	120	149	174	200
Respirations per minute	20	18	16	13	13	16	20	22

The rise in brain temperature after cooling was accompanied by restoration of normal circulatory and respiratory functions. As is evident from Table 1, arterial pressure rose practically to normal (98%, on the average) when brain temperature had risen to 33°, and in three experiments it was fully restored.

The heart rate increased rapidly, regaining its initial value when jugular vein temperature had risen to 35° (Table 2). Rise in respiratory rate and amplitude also accompanied rise in brain temperature.

It thus appears that the respiratory and circulatory indices studied by us were rapidly and almost completely restored to normal after profound cooling of the brain.

The skin incisions were stitched up after the temperature had returned to normal. The animals were given penicillin for a few days after the experiments, in order to prevent infection.

The cats were languid and inactive after the experiments, and lay down in normal attitudes; their sensitivity to pain was lowered, but they reacted to sound and light. Their condition was considerably improved by the next day, and they fed spontaneously. More prolonged observation of the animals did not reveal any deviations from the normal.

A hypothermic state can therefore be achieved by cooling the brain alone, through its external coverings. This procedure leads to a fall in both rectal and brain temperatures, but, in view of the direct application of cold

to the head, brain temperature falls faster and lower than rectal. We found similar effects [5] when the brain was cooled by extra-corporeal cooling of carotid artery blood. Using this procedure, we reduced brain temperature to 20°, when rectal temperature was about 30°. When the body as a whole is cooled, these temperatures are practically identical [11].

Cooling of the brain is achieved in our experiments by direct loss of heat from the interior of the brain to the cranium and scalp. As a result, the superficial parts of the brain, in the first place its cortex, lose heat faster than the underlying parts, so that its temperatures falls faster, and to a lower value, than in the lower levels. Such a differential gradient of temperatures in the brain and in the whole organism may be very convenient. More intense cooling of the cortical regions may lead to more profound inhibition of function than in the subcortical regions and the brain stem. In applying this procedure to intrathoracic operations involving exclusion of cardiac activity, for example, the most profound inhibition can be achieved in the cerebral cortex, which is the most sensitive to oxygen deficiency. The temperatures also fall in the lower levels of the brain, although to a smaller extent than in the upper ones. An even smaller fall in temperature is observed in the rectum. This may be ascribed to the absence of direct cooling of the trunk. The retention of a fairly high heart rate in the presence of profound hypothermia is a result of the relatively high temperature of the trunk, as compared with the brain. We found a similar effect when brain cooling was achieved by cooling carotid artery blood [5]. Apart from this, the maintenance of a fairly high body temperature, including the heart, eliminates the danger of development of ventricular fibrillation due to cold, while at the same time profound hypothermia of the brain is achieved. This feature may justify the adoption of this procedure in heart surgery, when it has been further investigated experimentally.

SUMMARY

The results of experiments with the isolated over-cooling of cats' brains are described. Deep hypothermia of the brain was achieved by its overcooling with the aid of "cold blankets". During such over-cooling the temperature of the brain is decreased more rapidly and more deeply than rectal. There is a difference in temperature between the external and internal portions of the brain. Thus, at the termination of over-cooling the temperature equalled, on the average: in the external layer of the brain (in the cortex) 18°C, in the medial layer (subcortex) 21.2°C, at the base of the brain 24.7°C and in the rectum 29°C.

Reduction of the brain temperature is associated with changes in respiration and circulation. The following increase of the temperature results in recovery of these functions. Later, no pronounced disturbances were observed in these animals.

The method of over-cooling of the brain through the external surface of the head may be used in surgical practice.

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DISTURBANCES OF VASCULAR PERMEABILITY IN SHOCK (ELECTROPHORETIC INVESTIGATION OF PROTEIN COMPOSITION OF THE LYMPH IN DEVELOPMENT OF PEPTONE SHOCK)

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It was shown in previous investigations [1, 2] that during peptone shock in dogs the elimination of intravenously administered labeled protein from the blood and its appearance in the lymph of the thoracic duct were accelerated. These data suggested that peptone shock was associated with changes in vascular permeability. Further elucidation of this question was undertaken with the help of studies on the protein composition of the blood and lymph during the development of peptone shock.

EXPERIMENTAL METHODS

Experiments were performed on dogs of both sexes and different weight. The femoral vein was dissected under morphine-ether anesthesia and used for injection of peptone solution; the femoral artery was used for recording blood pressure. A cannula was inserted into the thoracic lymph duct.

The blood and lymph for electrophoretic analysis were received into dry centrifuge tubes; the samples were collected before administration of peptone and 5 minutes after its injection.

Shock was produced by intravenous injection of 40% solution of peptone prepared with 0.85% sodium chloride solution, the dose being calculated to correspond to 0.7 g peptone per 1 kg body weight. Experiments were carried out on 17 dogs. Development of shock was monitored by measurement of blood pressure in the femoral artery. The blood pressure, as a rule, fell by $^2/_3$ of the initial value within 20-30 seconds after administration of peptone.

The total blood serum and lymph protein was determined by means of an immersion refractometer "IRF-1". Determination of the protein composition of the blood and lymph was carried out in part of the experiments by the electrophoretic method using the apparatus constructed in our laboratory, incorporating the optic arrangement proposed by G. V. Troitsky [6, 7]. Veronal buffer (pH = 8.6 at 20°; 0.1 M) was used. In many experiments the study of protein composition of the lymph by classic (free) electrophoresis was rendered difficult by insufficient transparency of the lymph. Therefore the majority of determinations were performed by means of filter-paper electrophoresis. Separation time – $4^1/_2$-5 hours with current strength 1 ma per 4 cm of paper width and voltage 7-8 v/cm, M = 0.46, pH = 8.6. The electrophoregrams were developed by 0.5% solution of bromphenol blue in concentrated alcoholic solution of mercuric chloride. The details of electrophoretic technic are described in the work of E. P. Smolichev [4, 5].

EXPERIMENTAL RESULTS

Statistic treatment of results obtained in 7 experiments with free electrophoretic determination of protein fractions showed that, in dogs, 5 minutes after the administration of peptone there was an increase in the concentration of protein in the lymph of the thoracic duct ($P < 0.001$), while the ratios of the protein fractions and

the value of the A/G coefficient remained unchanged ($P > 0.05$).

Statistically treated results of 10 experiments using filter paper electrophoresis are presented in Tables 1 and 2.

TABLE 1

Concentration of Protein (in Grams-Per Cent) and Ratios of Protein Fractions (in Relative Percentages) in Dog Blood Serum During Development of Peptone Shock

Statistic indices	Protein in g%	Albumin	Globulins					A/G
			α_1	α_2	β_1	β_2	γ	
			Before shock					
M	6.8	49.3	7.3	6.4	8.3	16.2	11.8	0.9
$\sigma\pm$	—	6.7	2.4	2.3	5.7	5.5	5.1	0.3
$m\pm$	—	2.1	0.7	0.7	1.1	1.7	1.6	0.09
			During shock					
M	6.6	51.4	7.1	6.4	6.3	15.6	13.0	1.1
$\sigma\pm$	—	8.2	1.4	2.8	2.7	7.7	7.7	0.35
$m\pm$	—	2.6	0.7	0.9	0.8	2.4	2.4	0.1

TABLE 2

Concentration of Protein (in Grams-Per Cent) and Ratios of Protein Fractions (in Relative Percentages) in Dog Lymph Serum During Development of Peptone Shock

Statistic indices	Protein in g%	Albumin	Globulins					A/G
			α_1	α_2	β_1	β_2	γ	
			Before shock					
M	3.4	61.2	6.2	5.1	4.7	8.9	12.7	1.6
$\sigma\pm$	1.1	7.7	2.9	2.7	1.6	4.0	6.6	0.6
$m\pm$	0.3	2.1	0.9	0.8	0.5	1.2	2.1	0.2
			During shock					
M	4.7	60.0	5.9	6.3	4.1	10.0	13.5	1.5
$\sigma\pm$	1.2	9.2	2.0	4.2	1.9	3.9	11.0	0.5
$m\pm$	0.3	2.9	0.6	1.3	0.6	1.2	3.5	0.2

Table 1 shows that 5 minutes after intravenous injection of peptone there were no changes ($P > 0.05$) in the concentration of protein, ratios of protein fractions and the A/G coefficient of blood serum in dogs.

Data presented in Table 2 demonstrate that 5 minutes after intravenous injection of peptone there is a rise in protein concentration of the lymph in the thoracic duct ($P < 0.001$) but no change ($P > 0.05$) in the ratios of protein fractions and the A/G coefficient.

DISCUSSION

Studies of capillary permeability by modern methods do not always reveal the development of general disturbances of permeability. Thus, M. Netsky and S. S. Leiter [11] found that intravenously injected horse serum protein appeared in the lymph of the thoracic duct more rapidly in dogs suffering from shock due to burns than in control animals. At the same time O. Cope and F. Moore [9], J. Fine and A. Seligman [10] demonstrated only local disturbances of capillary permeability in cases of shock due to burns. General disturbances of vascular permeability could not be demonstrated in rats suffering from hemorrhagic shock (R. Baratz and R. Ingraham [8]) and in dogs in ischemic shock (G. Szabo and L. Madyar [12]). In a recently published paper G. Szabo and L. Madyar [13] report that general disturbances of capillary permeability in histamine shock can be demonstrated in dogs but not in cats. Evidently, general disturbances of vascular permeability cannot be regarded as a state characteristic for all types of shock and underlying the pathogenesis of shock of various origin (I. A. Oivin [3]).

The present investigations show that in peptone shock the passage of proteins from the blood into the tissues is accelerated, the appearance of labeled protein in the lymph of the thoracic duct is also accelerated, the concentration of protein in the lymph is increased, but there is no change in the ratio of the blood and lymph protein fractions. The absence of change in the protein composition of the blood is easily explained. Under the influence of peptone lymph production and lymph flow are enhanced. The proteins are not retained in the tissues but enter the blood by way of the lymphatics. Therefore the concentration of protein in the blood does not alter. The enhanced passage of proteins from the blood into the lymph in peptone shock is not selective. All the protein fractions pass through the capillaries at the same rate. This is attested by the absence of changes in the ratio of the protein fractions in the lymph during development of peptone shock. There is thus no basis for speaking of selective disturbances of capillary permeability in peptone shock. The data obtained indicate an increase of overall [3] capillary permeability during the development of peptone shock.

SUMMARY

Peptone shock is developed in 5 minutes following the intravenous injection of peptone. There is an acute decrease in the blood pressure. There were no changes in the protein concentration and in the relationship between the protein fraction of the blood serum. A pronounced increase of the lymph flow with increased concentration of the protein in the lymph of the thoracic duct was noted. The average value of the protein concentration before the introduction of peptone equalled 3.5, $m \pm 0.11$ g%; following peptone introduction it was 4.7, $m \pm 0.2$ g%; ($P < 0.01$).

There were no changes in the relative content of the protein fractions of the lymph.

In dogs there is an increased permeability of the blood capillaries in peptone shock but they remain intact.

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THE ROLE OF INTEROCEPTORS IN THE REGULATION OF BLOOD COAGULATION

COMMUNICATION 2. THE ROLE OF VASCULAR RECEPTOR ZONES OF THE CAROTID SINUS AND THE ARCH OF THE AORTA IN ACCELERATION OF BLOOD COAGULATION IN ACUTE BLOOD LOSS

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The phenomenon of accelerated coagulation of blood in acute blood loss, which is a protective measure, has been described by many authors [2, 3, 5, 6, 10 and others]. It has been established that secretion of adrenalin by the suprarenals [1, 9 and others] is of great importance in this reaction, but the mechanisms have not until now received adequate study. Taking into account the practical value of investigation of protective mechanisms in blood loss, which is one of the most widespread forms of pathology in wartime and peacetime trauma, we undertook to investigate the part played by the carotid sinus and arch of the aorta interoceptors in accelerating coagulation of the blood in acute blood loss.

EXPERIMENTAL METHODS

Twenty-nine experiments were performed on rabbits and 10 on dogs. The experiments on rabbits were done without anesthesia, while those on dogs were performed under morphine-pentothal analgesia. In experiments on dogs kymographic records were made of respiration and of blood pressure in the femoral artery, (by means of a mercury manometer). The method for taking blood samples for determination of coagulation time was described in a previous communication [4]. Coagulation time of the blood was determined in a Bazaron apparatus placed in a water bath with a mercury-toluene thermoregulator which maintained the temperature in the apparatus at 36.5°. The thrombin titer was determined by the dilution method.

EXPERIMENTAL RESULTS

In the first series of experiments the effect of local carotid sinus anemia on blood coagulation was studied on 7 rabbits. A ligature was passed under the common carotid artery, after which the animal was allowed to rest for 1-1½ hours. In several cases the superficial dissection was carried out under local infiltration anesthesia (0.25% solution of novocain). After the interval the initial level of blood coagulation time was determined until comparatively constant results were obtained; this took as long as 2 hours in some experiments. When this initial level had been established the common carotid artery was tied off on one side. Following the ligation, coagulation time was estimated after 5 and 30 minutes. Definite acceleration of coagulation time was noted after 5 minutes in 6 out of 7 cases, no change being discernible in the case of 1 animal only. The average value of blood coagulation time was about 60% of the initial value (Fig. 1), i. e., was almost halved. By the 30th minute coagulation time approached the initial value despite the fact that the ligature was not removed from the artery. Adaptation of the carotid sinus receptors had evidently taken place.

N. N. Popova [8] observed acceleration of blood coagulation in dogs when both the common carotid arteries were ligated. Drawing an analogy with acute blood loss, Popova explains this by cerebral anemia and completely disregards the possible role of interoceptors in the genesis of this phenomenon.

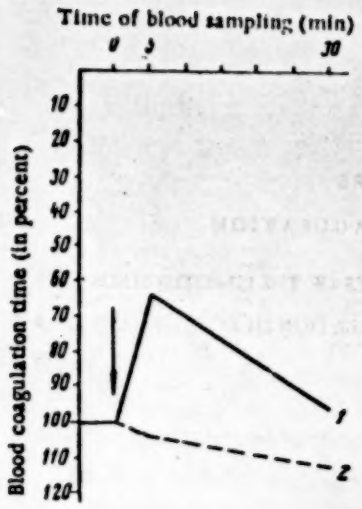

Fig. 1. Changes in blood coagulation time in rabbits following ligation of the carotid artery.
1) First series of experiments; 2) second series of experiments; | ligation of the carotid artery.

In order to determine whether the observed acceleration of blood coagulation was a reflex act or resulted from cerebral anemia, we staged a second series of experiments for control, in which rabbits were subjected to unilateral ligation of the internal carotid artery. The carotid sinus on the appropriate side was first denervated by section of the sinus nerve in order to prevent possible irritation of the sino-carotid zone. This excluded stimulation of the powerful vascular receptor zone while maintaining the same disturbances of cerebral hemodynamics as in the first series of experiments.

In this series of experiments, no reflex acceleration of blood coagulation was obtained (Fig. 1), and in 4 of the 7 experiments there was even some slowing of coagulation. These data agree with investigations [5] which had shown that anemia of various degrees could not be regarded as a physiologic regulator of autonomic centers and their activity and that cerebral anemia with preservation of normal carotid sinus reflexes led to a gradual diminution of activity in brain stem centers.

In the third series of experiments, 2 groups of rabbits had 6-10% of their total blood withdrawn; in the first group the sinus nerves were transected bilaterally. In the second group of animals the sinus nerves were left intact but incisions were made in the neck similar to those made in the first group. Withdrawal of blood with preservation of carotid sinus receptor zones was, as a rule, accompanied by more marked acceleration of blood coagulation than in rabbits with transected sinus nerves. This difference was especially well marked 5 minutes after withdrawal of blood when the difference of the means exceeded its error more than 3-fold; this testifies to sufficiently high reliability of the difference between the mean values. The results of this series of experiments are presented in Fig. 2. Some acceleration of blood coagulation after withdrawal of blood was, however, also noted in animals with transected sinus nerves. It must be taken into account that the depressor nerves were left intact in these experiments.

Dogs (which are more resitant to acute blood loss) were used in further experiments in which the question was studied in greater detail. The experimental animals were again divided into two groups: the first group served as control and included 4 dogs, the second consisted of 6 dogs with denervated carotid sinuses and transected vagi, including the depressor fibers. Blood coagulation was determined prior to withdrawal of blood; immediately afterward, and then after 10, 20, 30, 45 and 60 minutes. The amount of blood withdrawn corresponded to 2% of body weight. According to literature data [2] such blood loss is accompanied by considerable shortening of coagulation time and increased thrombin titer; this was confirmed in the present experiments. The results of this series of experiments are presented in Fig. 3. There is a clear difference between the change in blood coagulation time following withdrawal of blood in normal dogs and in dogs with "elimination" of receptors in the carotid sinus and arch of the aorta. In the case of dogs with preserved innervation the shortening of coagulation time was considerable, averaging over 50%, and was most pronounced by the 20th-30th minute after withdrawal of blood. In the case of dogs with transected sinus and vagus nerves acceleration of blood coagulation time was considerably less marked and developed mainly directly after withdrawal of blood then disappeared and was replaced by slowing. In these animals there was no increase in thrombin titer either.

The present experiments fully confirmed the data on the predominant role of carotid sinus and aortal nerves in the adaptation reaction of the cardio-vascular system to blood loss [11, 12 and others]. Compensatory cardiovascular changes are absent in animals with denervated carotid sinus and aortal zones; their blood pressure drops and does not recover following blood loss, whereas similar blood loss in control animals is associated with much less marked hypotension which is transient.

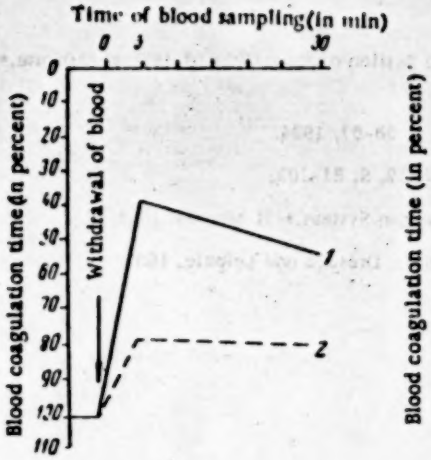

Fig. 2. Changes in blood coagulation time in rabbits following withdrawal of blood.
1) Experiments on normal rabbits; 2) experiments on rabbits with denervated carotid sinuses.

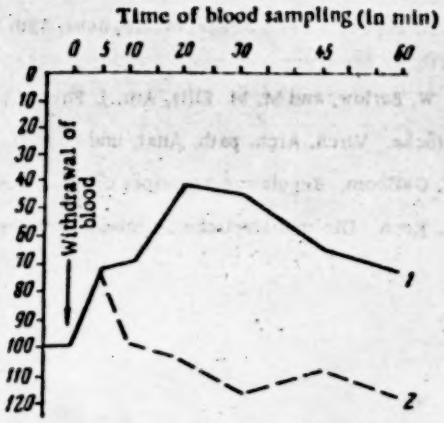

Fig. 3. Changes in blood coagulation time in dogs following withdrawal of blood.
1) Experiments on normal dogs; 2) experiments on dogs with denervated carotid sinus and aortal zones.

Acceleration of blood coagulation associated with excitation of the sympathetic-adrenalin system during acute blood loss is thus predominantly a reflex phenomenon dependent chiefly on the receptors on the carotid sinus and arch of the aorta. Together with hyperadrenalinemia, hyperglycemia, vasopressor reaction, tachycardia, hyperhydremia and other reactions accompanying acute blood loss, acceleration of blood coagulation is of great protective significance for the organism since it promotes cessation of hemorrhage.

Local anemia of carotid sinus zone elicits reflex acceleration of blood coagulation, while denervation of the carotid sinus and aortal zones impairs severely the protective acceleration of coagulation and rise of thrombin titer associated with acute blood loss.

SUMMARY

The role of interoceptors of carotid sinus and of the arch of aorta in acceleration of blood coagulation in acute blood loss was investigated. It was revealed by 29 experiments on rabbits and 10 on dogs that local anemia of sinocarotid zones causes reflex acceleration of blood coagulation. Blood loss in rabbits with denervated carotid sinuses is associated with the weaker response of acceleration of blood coagulation, than in normal animals. With exclusion of the sinus, depressor and vagus nerves the acceleration of the blood coagulation and increase of thrombin titer is not as considerable as following blood loss in the intact animals.

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THE MECHANISM OF INTOXICATION IN DYSENTERY

COMMUNICATION 2. THE EFFECT OF DYSENTERY TOXIN AND ANATOXIN ON INTESTINAL RECEPTORS

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The results of experiments described in the first communication [1] showed that the action of the Shiga dysentery toxin on the mucosa of a perfused section of the intestine of an animal poisoned with the same toxin produced changes in the intensity of reflexes from intestinal chemoreceptors.

It remained unclear whether these changes were caused by the action of the toxic group of the toxin or by enhanced sensitivity to its antigenic group. The present work is concerned with elucidation of these questions.

EXPERIMENTAL METHODS

A total of 26 experiments was carried out on cats; in 5 of these the animals had had a preliminary administration of anatoxin, in 15 the animals were in a state of dysentery intoxication and in 6 the animals had been immunized by dysentery toxin 3 days prior to the experiment. Anatoxin was prepared by addition of formalin to the toxin used.

In the first series of experiments the animals received anatoxin subcutaneously (24 hours prior to the experiment) in doses corresponding to 20 lethal mouse doses per 1 kg body weight. In one experiment the dose of anatoxin corresponded to 40 lethal doses of toxin per 1 kg weight.

Two groups of animals were used in the second series of experiments. The first group (6 experiments) was given toxin subcutaneously 4-6 hours prior to the experiment, the second group (9 experiments) was given the toxin subcutaneously 24 hours prior to the experiment.

The third series of experiments was performed on animals which had been immunized by five administrations of anatoxin at 5 day intervals and in amounts corresponding to 60 lethal doses of the toxin (14-17 lethal doses per 1 kg body weight). Fourteen days after the last dose of anatoxin the animals were given 30 lethal doses of toxin subcutaneously on two consecutive days (a total of 14-17 lethal doses per 1 kg body weight). A critical experiment was performed on the fourth day.

As in the previous experiments, perfusion of an intestinal loop was used in the present work. The toxin and anatoxin were introduced into the lumen of the perfused segment of the intestine.

The order of experimental procedures remained as before [1].

EXPERIMENTAL RESULTS

In all the 5 experiments of the first series introduction of anatoxin into the lumen of the intestine exerted no effect on the pressor reactions elicited by subsequent introduction of nicotine into the vessels.

In four experiments introduction of anatoxin was followed by application of toxin to the intestinal mucosa. In one of these experiments a slight increase in the reflex blood pressure reaction was noted (a rise of 2 mm of Hg). In the remaining experiments the reflexes were unchanged. Increase of arterial blood pressure was elicited in these experiments, as in the case of healthy animals, by the addition of 1 ml nicotine in dilution 1×10^{-6} (1 γ) to the perfusion fluid.

At autopsy no changes were found in the internal organs.

Figure 1 shows the kymograms of one of these experiments. The initial stimulation of the intestinal loop receptors by nicotine (4 γ) gave rise to an increase in arterial blood pressure equal to 6 mm Hg. Introduction of anatoxin (⌊¹) into the intestinal lumen did not cause a rise in blood pressure or stimulation of respiration. The magnitude of reflexes to nicotine following introduction of anatoxin remained as before: 6-8 mm Hg (b). Reflexes of the same magnitude were obtained in the course of 52 minutes following introduction of the anatoxin.

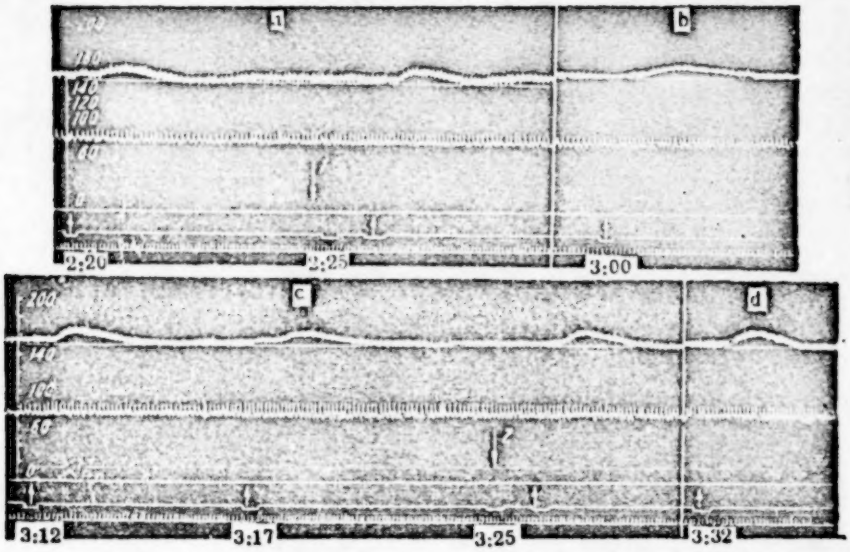

Fig. 1. Reflex reactions in a cat in experiment with preliminary administration of anatoxin in dose corresponding to 18 doses lethal for mouse per 1 kg body weight. Records from above down: arterial blood pressure, initial level of arterial blood pressure, respiration, base line of manometer, stimulus marker, time marker (2 seconds).

⌊¹ – administration of anatoxin, ⌊² – administration of toxin, ⌊ – nicotine 4 γ.

Application of toxin to the intestinal mucosa (⌊²) caused no change in the magnitude of reflex blood pressure and respiration reactions.

It could thus be concluded, on the basis of this series of experiments, that introduction of anatoxin as well as of toxin into the intestinal lumen of animals which had previously received anatoxin did not lead to changes in pressor reactions of arterial blood pressure in response to introduction of nicotine into the vessels.

Consequently, those changes which were obtained when toxin was given to animals which had been previously given the same toxin subcutaneously were caused by the state of dysentery intoxication and not by the action of the antigen group of the toxin.

The second series of experiments was carried out on animals in a state of dysentery intoxication. Six experiments in this series were performed on animals which received toxin 4-6 hours prior to the experiment (20-30 lethal mouse doses per 1 kg body weight). The remaining 9 animals were taken for experiment 24 hours after receiving the toxin. The dose was 15-30 lethal mouse doses per 1 kg body weight.

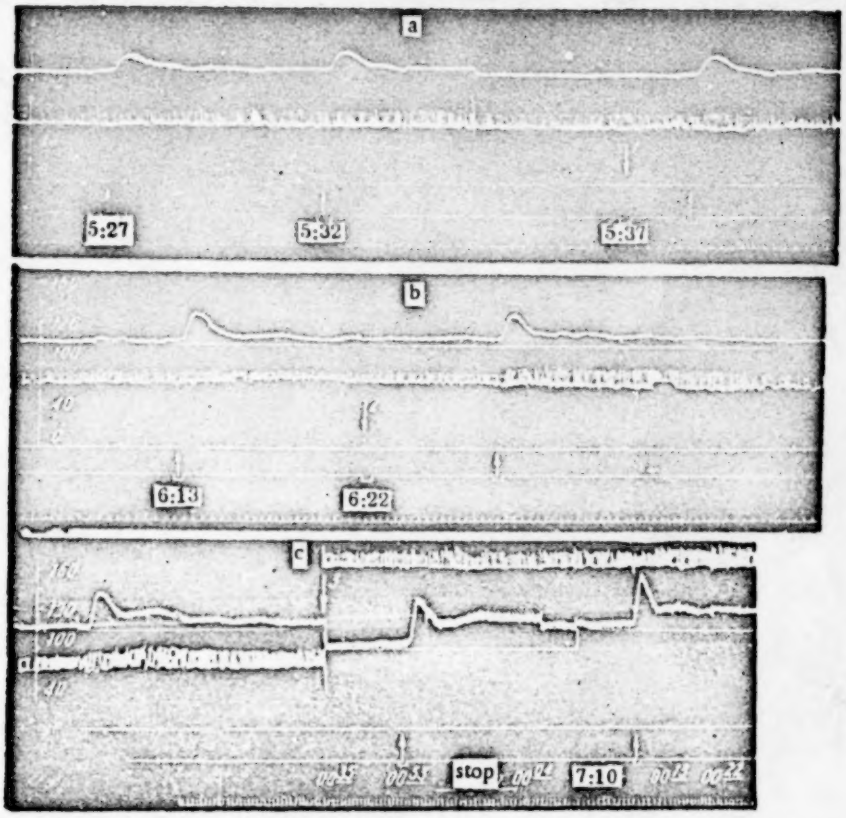

Fig. 2. Reflex reactions in a cat following preliminary administration of dysentery toxin ($\downarrow^{3}$) (35 lethal mouse doses per 1 kg body weight).
Records the same as in Fig. 1. Time marker (5 seconds), $\downarrow$ – introduction of nicotine 40 γ.

Introduction of anatoxin into the lumen of the perfused intestinal loop did not change the reflex arterial blood pressure reaction to nicotine introduced into the blood vessels of the same loop in a single one of the 6 experiments. Since the magnitude of the reflexes remained unchanged, 30-40 minutes later toxin was introduced into the intestinal lumen. In 4 experiments this led to increase of reflexes to nicotine as compared to the initial values. In two experiments there was no change in reflexes to nicotine. No changes were found in the internal organs of these animals at autopsy.

The changes described above are presented in Fig. 2. Introduction of 40 γ nicotine into the vessels of the intestinal loop was accompanied by reflex rise of arterial blood pressure by 14 mm Hg. Following application of anatoxin ($\downarrow^{1}$) to the mucosa of the perfused loop the reflexes remained unchanged over a period of 30 minutes. After 30 minutes (following introduction of anatoxin) the reflexes to nicotine rose to 24 mm Hg with an unchanged level of arterial blood pressure. It is unlikely that this rise could be connected with the action of anatoxin since usually restoration of altered reflexes was observed after 30-40 minutes. Against this background toxin was given ($\downarrow^{2}$) (120 lethal doses) 45 minutes after the introduction of anatoxin. Nicotine was given 1 minute after the toxin

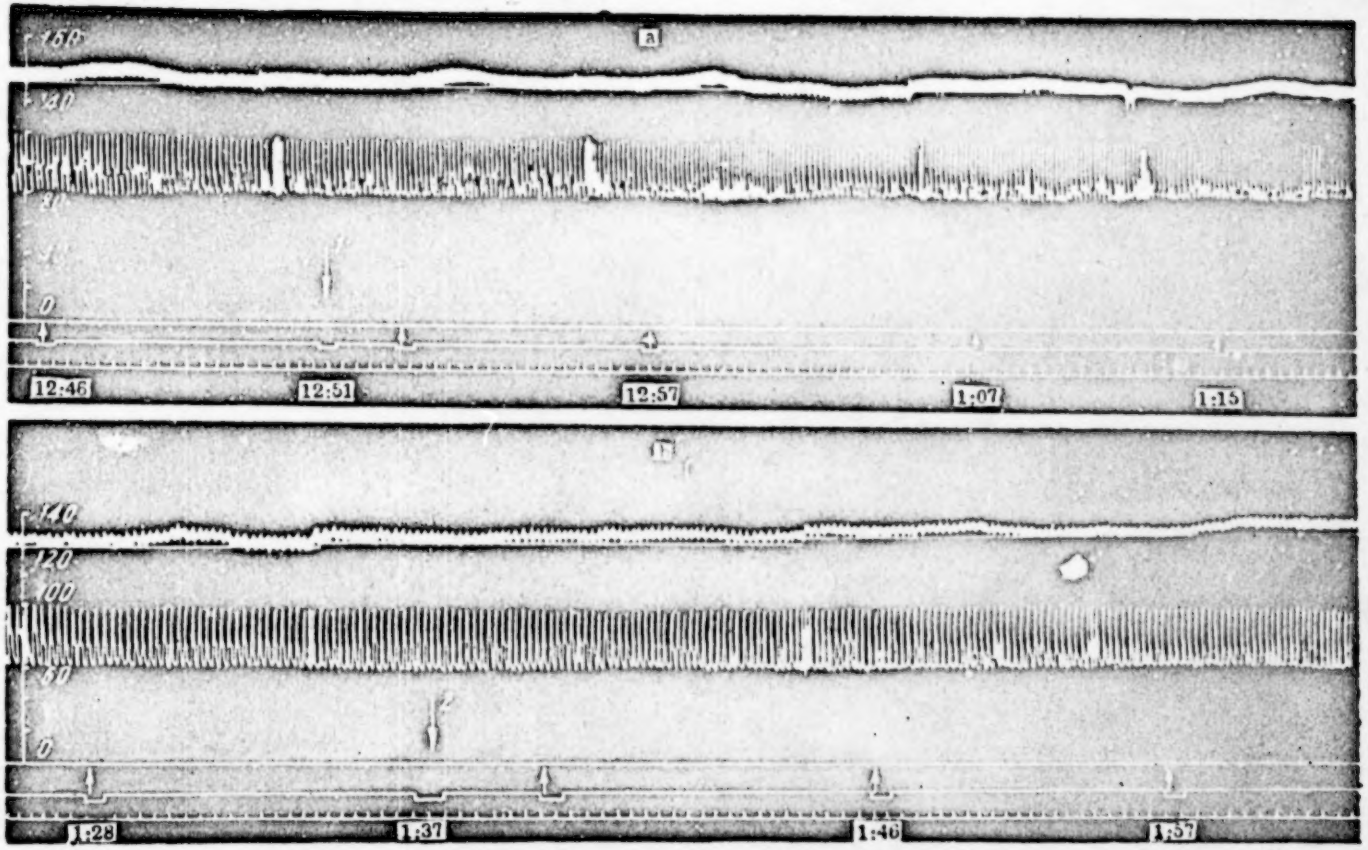

Fig. 3. Reflex reactions in a cat immunized with anatoxin (toxin given in the dose of 17 lethal mouse doses three days prior to the experiment). Records as in Fig. 1. Time marker (5 seconds), ↑ – administration of nicotine 4 γ.

and elicited a prolonged rise of blood pressure of the same magnitude. In 10 minutes the reflex to nicotine reached 32 mm Hg, exceeding by 8 mm Hg the level of the initial reflex. Deepening of narcosis ($\downarrow^1$) did not lower the magnitude of the reflexes. The experiment was completed 1 hour after administration of toxin. The reflex rise of arterial blood pressure to administration of nicotine was long lasting and reached 45 mm Hg, exceeding the level of arterial blood pressure increase prior to application of toxin to the mucosa of the intestinal loop by 21 mm.

In 9 experiments of this series, performed on the second day of intoxication, enhancement of reflex reactions to nicotine under the influence of anatoxin was observed in one case only. In the remaining 8 experiments the reflexes were unchanged.

This whole series of experiments gives grounds for concluding that the state of intoxication does not alter pressor reactions of arterial blood pressure to nicotine following introduction of anatoxin into the lumen of the intestinal loop.

It can therefore be postulated that in dysentery intoxication the sensitivity of intestinal chemoreceptors to the antigen group of the toxin remains unchanged while their sensitivity to the toxic group increases.

The third series of experiments (6 experiments) was carried out on animals in which the dysentery toxin was introduced against the background of antitoxic immunity. The animals' behavior revealed no signs of intoxication. At autopsy no changes in internal organs were found in 5 of the 6 animals. Only in one animal, which had received a very large dose of toxin (43 lethal mouse doses per 1 kg body weight), there was slight edema and hyperemia of the mucosa in the proximal part of the large intestine. In the same case application of toxin to the mucosa of the intestinal loop was accompanied by enhancement of reflexes to nicotine (10 γ and 4 γ). In the other 5 experiments introduction of toxin into the intestinal lumen did not lead to any changes of the pressor reactions of arterial blood pressure.

Figure 3 shows kymograms of an experiment in this series. Throughout the whole experiment (over one hour) the initial magnitude of the reflex reactions to 4 γ nicotine (4 mm Hg) was maintained despite two introductions of the toxin into the intestinal lumen ($\downarrow^1$, $\downarrow^2$).

In this series of experiments the threshold to nicotine stimulation was above 1γ(in 2 experiments – 4 γ, in 4 experiments – 10 γ). Such lowering of sensitivity to a nonspecific stimulant (acetylcholine) in the presence of immunity has been noted previously by G. Rashkova and co-workers [2].

In the latter experiments the repeated introduction of anatoxin could create conditions of sensitization to dysentery antigen. However, application of dysentery toxin to the intestinal mucosa in these experiments did not affect the magnitude of reflex reactions to nicotine. This can, evidently, serve as proof of lowered sensitivity of intestinal chemoreceptors to dysentery toxin in immunized animals, which is also manifested by the absence of clinical signs of the disease.

Similar lowering of sensitivity of intestinal vascular receptors to dysentery antigen was obtained by A. G. Skavronskaya [3].

Experiments on cats reported by G. Rashkova and collaborators also revealed lowering of sensitivity of receptors in intestinal vessels to the Shiga dysentery toxin in immunized animals [2].

The present investigations give grounds for stating that substantial differences exist between the action of the antigen and toxic groups of the toxin on intestinal receptors.

While in the state of intoxication the sensitivity of receptors to the dysentery toxin is apparently increased [1], this sensitivity is lowered in antitoxic immunity.

It is possible that lowering of receptor sensitivity to the toxin in immunized animals determines to some extent the fact that these animals are not susceptible to the disease under the influence of the specific pathogen.

SUMMARY

In condition of dysenteric intoxication, changes of reflexes from the chemoreceptors of intestines are due to the toxic, not the antigenic group of the toxin. In animals immunized by dysenteric anatoxin the sensitivity of intestinal receptors to dysenteric toxin is decreased.

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** In Russian.

MECHANISM OF THE ACTION OF STAPHYLOCOCCUS ENTEROTOXIN ON THE ANIMAL BODY

COMMUNICATION 2. THE EFFECT OF STAPHYLOCOCCUS ENTEROTOXIN ON CIRCULATION

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In Communication 1 results of our experiments on the effect of staphylococcus enterotoxin on the motor function of the stomach and intestine of animals were presented. These experiments showed that the cholinergic effect of staphylococcus enterotoxin played an important part in the mechanism of its action on the digestive organs.

The presence of vagotropic action shown by staphylococcus enterotoxin with respect to the intestine gave immediate grounds for supposing that it would have an active effect on the circulatory apparatus.

Further reactions occurring in the body under the influence of enterotoxin filtrates from staphylococci on the circulatory apparatus were investigated. The experiments were performed on 71 cats. Our investigations were confined to an attempt to solve a limited problem within the complex of reactions occurring under these conditions; this limited problem posed the question whether the circulatory apparatus was affected in staphylococcus enterotoxin poisoning and what were the basic mechanisms of such involvement.

This problem had not received experimental investigation.

EXPERIMENTAL METHODS

The first series of experiments was concerned with the effect of staphylococcus enterotoxin on the arterial blood pressure of intact animals. A total of 22 experiments on 17 cats was carried out.

The experimental method consisted of recording arterial blood pressure in cats, under urethane anesthesia, before and after intravenous administration of staphylococcal enterotoxin filtrates. The pressure in the common carotid artery was recorded by a spring manometer, and in 6 cases by a mercury manometer. Respiration was recorded by means of a Marey's tambour.

Staphylococcal enterotoxin filtrates were introduced (in doses of 2 ml/ kg) into the jugular vein which, like the common carotid artery, was exposed prior to the experiment.

The filtrates tested were of those staphylococcal strains which produced food poisoning and enterotoxic syndromes when tested on kittens and pups. Sterile nutrient medium and filtrate of staphylococcal strain which did not possess enterotoxic properties were used as controls.

EXPERIMENTAL RESULTS

A definite arterial blood pressure reaction was observed upon intravenous administration of staphylococcal enterotoxin filtrates in doses of 2 to 6 ml.

Immediately after injection of the enterotoxin filtrates there was a considerable drop in blood pressure, lasting up to 24 minutes and followed by a subsequent levelling out. In some experiments, mainly when large doses of the filtrates were given, a second fall in blood pressure occurred and in many cases resulted in the animal's death. The average fall of systolic pressure was 43 mm Hg, of diastolic pressure – 36 mm. The rate of cardiac contractions was increased in the majority of the experiments. Duration of the drop in blood pressure was, in some cases, over one hour. The blood pressure did not return to the original level in any of the experiments (Fig. 1).

In addition to a fall in blood pressure and acceleration of the cardiac rhythm changes in respiration were observed in most of the experiments. The respiratory excursions increased in frequency with a simultaneous diminution in amplitude; this occurred most frequently immediately after injection of the enterotoxin filtrates. The amplitude of respiratory movements began to increase towards the end of the experiments.

Seven control experiments showed that sterile nutrient medium, used for growing the staphylococcal enterotoxin, and filtrates of staphylococcal strains without enterotoxic properties produced a rise of arterial blood pressure in the experimental animals.

The fact that intravenous injection of sterile nutrient medium caused a rise of arterial pressure lends further support to the conclusion that the observed drop in blood pressure was undoubtedly associated with the action of staphylococcal enterotoxin filtrates.

It is known from clinical observations that facial pallor and lowering of blood pressure are associated with cases of food poisoning of staphylococcal origin. The vasomotor reaction and fall of blood pressure in these patients are evidently connected with the action of staphylococcal enterotoxin on the circulatory apparatus. This is fully confirmed by our experimental studies of this question. However, this does not provide a definite answer as to the mechanism of the reaction since it could arise from: 1) impairment of central regulation of blood circulation (collapse), 2) involvement of peripheral blood vessels (by analogy with the action of arsenic) and 3) impairment of cardiac activity.

Experiments on cats following administration of adrenalin, atropine and papaverine were carried out in order to elucidate the mechanism of blood pressure lowering caused by staphylococcal enterotoxin filtrates. In all the experiments the blood pressure was recorded by means of a mercury manometer.

Six experiments were staged against the background of adrenalin action. 1 ml/ kg adrenalin in concentration 1:10,000 was given intravenously and the reaction obtained was similar to that observed in intact animals: fall of blood pressure and acceleration of the cardiac contraction rate.

The response to enterotoxin filtrates against the background of adrenalin action, which produced enhanced sensitivity of organs and tissues to adrenergic reactions, was thus similar to that obtained in the preceding series of experiments on intact animals. Had the staphylococcal enterotoxin filtrates affected adrenergic reactions inversion of the response could have been expected.

The next 6 experiments were performed on atropinized cats. Atropine was given intravenously in the dose of 0.0005 g per 1 kg body weight in the form of a 0.1% solution of atropine sulfate (0.5 ml/ kg). As in the preceding experiments, a fall in blood pressure and acceleration of the rate of cardiac contractions were observed.

It should be noted that, in comparing the blood pressure values, in two cases there was even an increase (experiments Nos. 5 and 6), while in the remaining experiments, except No. 1, there was a slight lowering of blood pressure averaging 30 mm Hg, i. e., less than in the experiments with adrenalin cited above (46-39 mm Hg).

The experiments in which adrenalin and atropine were used pointed to a definite relation between blood pressure and the activity of the central sections of the autonomic nervous system. The results of these experiments thus do not contradict the proposed hypothesis but do not provide direct proof of it. Four more experiments were therefore carried out, with administration of papaverine as a myotropic agent.

Intravenous administration of staphylococcal enterotoxin filtrate against the background of papaverine action was followed by appreciable fall in blood pressure. The systolic pressure fell by an average of 40 mm Hg, the diastolic – by 31 mm; in experiments with adrenalin the systolic pressure fell by an average of 46 mm, diastolic – by 39 mm, and in those with atropine the former fell by 30 mm on the average.

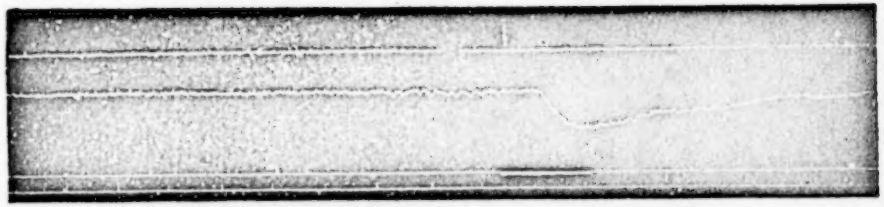

Fig. 1. Changes in blood pressure under the influence of staphylococcal enterotoxin filtrate. Kymogram of experiment No. 50.
Records from above down: 1) respiration; 2) blood pressure; 3) blood pressure base line; 4) time marker (20 seconds); | – enterotoxin injection.

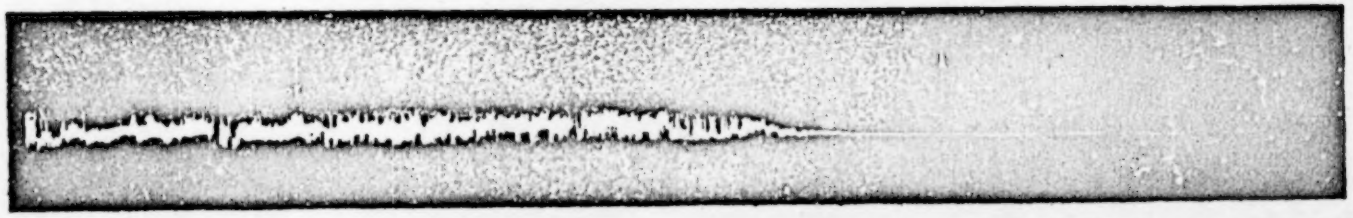

Fig. 2. Changes in blood pressure under the influence of staphylococcal enterotoxin filtrate on the isolated heart. Kymogram of experiment No. 7; | – enterotoxin injection, | – washing.

In subsequent experiments the influence of enterotoxin filtrates on the arterial blood pressure of cats was determined after preliminary transection of the spinal cord.

In all 4 experiments the staphylococcal enterotoxin filtrates produced lowering of blood pressure but to a lesser extent than in experiments on animals with intact spinal cord.

These data not only do not contradict but definitely support the working hypothesis of vagotonic influence of these filtrates.

It is known that parasympathetic impulses are excitatory with respect to hollow smooth-muscle viscera of the gastro-intestinal tract and inhibitory with respect to the smooth muscle elements of the vascular wall.

The series of experiments on animals pretreated by various autonomic poisons (atropine, adrenalin, papaverine) prior to administration of staphylococcal enterotoxin filtrates thus showed that the latter maintained their characteristic action under these conditions, causing hypotension in the experimental animals.

As already mentioned, the extent of blood pressure lowering was least marked in the atropinized animals. These findings suggested that preservation of the animal's reaction to administration of enterotoxic filtrates after treatment with autonomic poisons could depend on the ability of the organism to mobilize its compensatory adaptation.

Since the administration of autonomic poisons could be associated with changes in the function of some organs leading to changes in the activity of others, it was decided to stage the experiments under more "pure" conditions, using the classic isolated heart method.

The experiments were performed on hearts isolated by the Kravkov technic. The animals used were adult cats. Following isolation, the heart was perfused for 15-25 minutes with Ringer-Locke solution warmed to 37°. When a stable background of activity was established, the heart was switched to perfusion with staphylococcal enterotoxin filtrates in dilutions of 1:50-1:100. A total of 14 isolated heart experiments was carried out.

It was found that perfusion of the isolated cat heart with a solution of staphylococcal enterotoxin filtrate led to rapid cardiac arrest in diastole. Such arrest occurred in 11 of the 14 experiments, while bradycardia accompanied by diminished strength of cardiac contractions was observed in 2 experiments.

Subsequent washing of the heart with nutrient medium failed to restore its activity in 6 cases and led to transient resumption of activity in 4 cases. No washing was performed in 4 cases (Fig. 2). In 6 of the 7 control experiments the amplitude of cardiac contractions showed some increase under the influence of sterile nutrient medium. The rate of cardiac contractions increased in 5 cases.

The staphylococcal enterotoxin is thus characterized by marked toxicity with respect to the isolated heart, causing its arrest in high dilution in Ringer-Locke solution.

Consequently, the hypotensive action of staphylococcal enterotoxin may be attributed to the depressive effect of the enterotoxin both on the vasomotor centers and the heart muscle.

SUMMARY

The effect of staphylococcus enterotoxin on the circulatory apparatus was investigated. Experiments were conducted on cats. It was established that there is a fall in the blood pressure in intravenous injection of staphylococcus enterotoxin. Experiments which were carried out on animals in which atropine, adrenalin and papaverine were previously administered showed that in these conditions enterotoxin, likewise, causes hypotonia.

It was also demonstrated that the isolated heart is arrested in the diastolic phase under the effect of staphylococcus enterotoxin.

The results of these experiments allow us to conclude that the effect of staphylococcus enterotoxin is mostly manifested by way of the elements of the parasympathetic portion of the nervous system.

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* Original Russian pagination. See C.B. Translation.

THE EFFECT OF SPINAL CORD TRANSECTION ON RENAL WATER EXCRETION

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In contrast to the views held by Verney [4] concerning strictly local distribution of osmoreceptors along the course of the internal carotid artery in the hypothalamic area, we suggest that osmoreceptors, sensitive to osmotic changes, are widely represented in the body, as has been shown by V. N. Chernigovsky [3] for baro – and chemoreceptors. It can be expected, on the strength of this suggestion, that substantial changes in the rate of excretion of water supplied in excess would accompany transection of the spinal cord isolating to a considerable degree the tissue osmoreceptors from the hypothalamic centers. The present work is devoted to the experimental verification of this suggestion.

EXPERIMENTAL METHODS

The experiments were performed on dogs with exteriorized ureters and on rats with urinary bladder fistulas.

Hydration of the animals was calculated on the basis of 5% of body weight.

In a number of experiments renal filtration was determined by the inulin clearance method and concentration of solid substances in the blood plasma by refractometric methods.

EXPERIMENTAL RESULTS

When control dogs were given 50 ml water per 1 kg body weight there was considerable increase in diuresis. The diuresis sometimes reached 8-10 ml per minute per 1 m^2 surface area. As a rule the animals excreted the water almost completely in 2-3 hours.

Following transection of the spinal cord at the level T4-T5 no water diuresis developed at all. As can be seen from a typical experiment (Fig. 1), only 19.5% of the administered fluid was excreted in 175 minutes.

Such a sharp drop in the rate of water excretion may be determined by various factors.

First of all slowing of absorption from the intestine may be postulated. This suggestion is, however, excluded, by investigation of changes in the concentration of blood plasma solids following hydration.

In intact animals the maximal drop in plasma solids following hydration developed after 60 minutes and did not exceed 7-12%. The refractometric index of the plasma returned to normal by the end of three hours.

Data of the control experiments corresponded entirely to the results obtained by L. I. Kurdyban [2] and V. I. Inchina [1].

The refractometric index of the plasma in operated animals decreased more rapidly and remained low for a longer time. In their case hydremia reached a maximum after 30 minutes, the index fell by 24-26% of the original value and showed no tendency to rise, remaining at a low level for over 3 hours.

Apparently, in the case of dogs with transected spinal cord the water is absorbed at the usual rate but is excreted extremely slowly.

This raised the question whether the diminution of renal excretory function upon transection of the cord was determined by lowering of arterial blood pressure and the associated reduction of filtration.

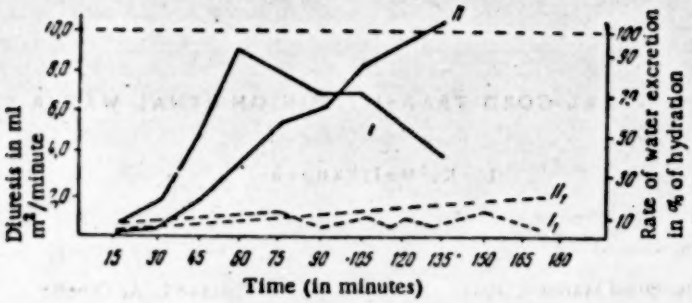

Fig. 1. Reaction to hydration of normal dogs (solid line) and dogs with spinal cord transection at the level T4-T5 (broken line).
I) Change in diuresis; II) percentage renal water excretion.

Determinations of renal filtration capacity by means of the inulin clearance test carried out on rats provide grounds for rejecting such an explanation of the observed phenomenon.

The results of this series of experiments are given in the table (mean values).

Changes in Renal Diuretic Activity Following Transection of the Spinal Cord

Animals under investigation	Number of experiments	Excretion of urine in 1 hour per 100 g body wt., ml	Excretion of water in % of amount administered, in 3 hrs.	Filtration in 1 hour per 100 g body wt., ml
Control rats	20	2.2	90	1.1
Rats with transected spinal cord	18	0.8	46	1.3

The value of inulin clearance in individual experiments is subject to definite individual fluctuations. However, these fluctuations in the operated animals are no different from fluctuations in control animals, whereas the average magnitudes of filtration in both groups practically coincide. The decline in water excretion following operation is, apparently, not connected with impairment of glomerular function.

It remains to suggest that the prolonged retention of excessive water in the body following spinal cord transection is associated with interruption of the afferent part of the osmoregulatory reflex arc.

Under normal conditions this reflex inhibits secretion in the neurohypophysis. Diuresis follows hydration as the direct result of the fact that antidiuretic hormone ceases to pass into the blood. Under conditions of spinal transection, however, the stream of afferent impulses from the tissue osmoreceptors to the hypothalamus is interrupted, and hyperhydration of the organism does not lead to reflex inhibition of pituitary activity as is the case in the normal. There is, consequently, no reduction in the concentration of the antidiuretic hormone in the blood and diuresis is not enhanced.

Such an explanation of reduced rate of water excretion agrees well with the fact that the degree of impairment of renal water excretory activity depends on the level at which the cord is transected. The higher the transection, the smaller the diuresis that develops. Thus, when the transection is made in the upper lumbar region approximately 70-80% of administered water is excreted, while the percentage excreted after transection at the level T4-T5 fluctuates within the range of 18 to 30.

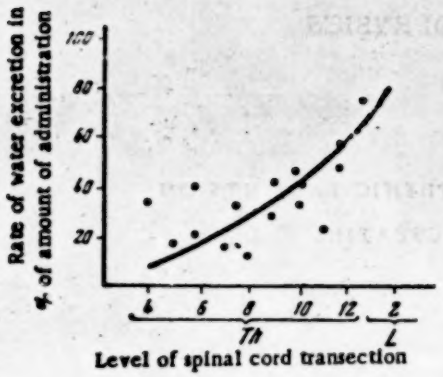

Fig. 2. Relation of the degree of reduction in the rate of excretion of 5% hydration load to the level of spinal cord transection.

The results of these experiments are summarized in Fig. 2. The curve of this graph is plotted on the basis of data obtained from all the experiments, both on dogs and on rats.

Although several of the experiments deviate from the regular course of the curve, it is still clear that water excretory function deteriorates at higher levels of spinal cord transection.

It has thus been established experimentally that following spinal cord transection in dogs and rats water diuresis cannot develop to any significant extent and the excessive amount of water is excreted slowly. This phenomenon cannot be explained by slowed absorption of water from the gastro-intestinal tract or by decreased filtration in the glomeruli.

The reason for deterioration of renal function lies in insufficiency of the osmoregulatory reflex as the result of interruption of afferent pathways from hyperhydration sensitive tissue receptors to hypothalamic nuclei.

SUMMARY

It was established that section of the spinal cord brings about decreased water diuresis. The higher the level of the section the greater the development of water diuresis. This phenomenon is not connected with delay in the water absorption from the gastro-intestinal tract, or with decreased filtration in the kidney glomeruli. Disturbance of water excretory function of the kidney following section of the spinal cord is connected with the break of the afferent routes from the osmoreceptors to the nuclei of the hypothalamic area.

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BIOCHEMISTRY AND BIOPHYSICS

THE EFFECT OF SERUM FROM MYASTHENIC PATIENTS ON FORMATION OF PHOSPHOCREATINE

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The pathogenesis of myasthenia, a severe neuromuscular disease, has not as yet been exhaustively investigated. In all probability myasthenic disorders are not fully accounted for by impairment of synaptic transmission.

A number of investigations have shown that myasthenia is accompanied by impairment of tissue respiration. N. I. Grashchenkov, L. A. Blumenfeld, L. B. Perelman, S. E. Krasovitskaya et al [1] discovered that during the period of myasthenic cachexia the O_2 content of venous blood was increased; administration of proserine* was followed by an appreciable drop in venous blood oxygen which was, evidently, taken up by the tissues. Indirect evidence for this is also provided by oxyhemometric examinations (Rivin) which showed that following administration of proserine there was a decrease in oxygen saturation of arterial blood.

Studies on creatine-creatinine metabolism also furnish evidence for impairment of oxidative processes in the muscles. Creatine may be found in the urine of myasthenic patients. A certain correlation could be observed between the dynamics of urinary creatine and changes in the clinical condition of the patients. According to our data, urinary creatine is only found in patients with marked myasthenic disturbances. Improvement in the clinical state of the patients was often accompanied by diminished excretion of creatine.

The following data can also serve as evidence for changes in tissue respiration in myasthenia. Proserine, a cholinesterase inhibitor, causes accumulation of acetylcholine which, in myasthenia, improves neuromuscular transmission. This is accompanied by accumulation of adrenalin-like substances in the blood. The improvement in the state of myasthenic patients is thus associated with increasing content of adrenalin-like factors which activate respiratory and glycolytic processes in the tissues.

The present investigation is concerned with the study of the effect of myasthenic blood on certain processes of tissue respiration. The working hypothesis adopted was that which suggested the possible presence in the blood of myasthenic patients of a factor eliciting myasthenic disorders. It is known that Torda and Wolff [2] showed that myasthenic serum depresses acetylcholine synthesis. H. Schwarz [3] points out that when serum from myasthenic patients is given to healthy subjects the latter develop muscular weakness. A. Wilson and H. Stoner [4] showed that normal serum did not affect the excitability of the neuromuscular preparation and tetanus values whereas serum from myasthenic patients depressed both the former and the latter. A depressing effect on excitatory processes is also exerted by thymus gland extracts from myasthenic patients but not by such extracts from healthy subjects [5]

METHODS

Oxidative phosphorylation was studied on ground rat muscle which was incubated with phosphate buffer at pH 7.8 in the presence of creatine.

* Russian trade name.

Composition of phosphate buffer: 1.15% KCl; 0.15 M $Na_2HPO_4 \cdot 2H_2O$; 3.82% $MgSO_4 \cdot 7H_2O$; 1.3% $NaHCO_3$. To 2 volumes of this buffer was added 1 volume of NaCl 0.9% and creatine calculated to correspond to 0.15%.

Composition of samples: 3 ml freshly prepared buffer, 0.5 ml serum, 300-500 mg muscle. Incubation – 30 minutes at 30°.

The reaction was terminated in the cold by precipitation with 3 ml ice-cold 5% trichloracetic acid. Inorganic phosphate was precipitated in the cold by an equal volume of magnesia mixture and phosphocreatine phosphorus was determined in 1 ml of centrifugate on incubation with ammonium molybdate. In investigating the effect of boiling 0.5 ml of serum was placed in a flask and brought to the boil before the addition of the remaining reagents to the same flask. The effect of dialysis was determined by preliminary dialysis of 3 ml serum against 3 ml NaCl 0.9% in the cold over a period of 24 hours followed by further determination on 0.5 ml of the dialysate. Respiration was determined in a Warburg apparatus on the same samples.

The rate of phosphocreatine formation served as an index of oxidative phosphorylation. The influence on this process of healthy and myasthenic serum, before and after administration of 2 ml 0.05% proserine to the patient, was studied.

RESULTS

In the first series of investigations the effect of serum from a healthy subject on phosphocreatine formation was studied. It was found that such serum exerted a depressing effect on the process. The degree of such depression is not a constant value but varies within wide limits, depending on the peculiarities of the muscle and rate of phosphorylation under given conditions. Several results of investigation of the effect of serum on phosphocreatine formation are presented in Table 1.

TABLE 1

Phosphocreatine Formation (in γ P per whole sample)

Without addition of serum	With added serum from healthy subject
560	470
425	352
230	105

Depression does not depend on the possible presence of phosphatases in the blood since this depressing effect on phosphorylation was preserved following boiling and dialysis. The factor exerting an inhibitory effect on phosphocreatine formation could be some substrate capable of intercepting part of the adenosinetriphosphate (ATP) phosphorus and so lead to diminished formation of phosphocreatine. Glucose, for example, could be such a substrate. In fact, addition of glucose (instead of serum) in amounts corresponding to its content in blood to the sample in which phosphorylation is taking place also leads to depression of phosphocreatine formation. The following distinctive feature was established: a 2-3 fold increase or 2 fold decrease of the amount of added glucose did not result in a definite change in the degree of phosphorylation depression.

In the next series of experiments a comparative study was made of the effect of myasthenic serum and of donor blood (control) on phosphocreatine formation. In each experiment a parallel and simultaneous investigation of the effect of myasthenic and healthy serum was carried out. Absolutely identical experimental conditions were thus maintained for the blood being tested and for donor blood. In each experiment the value of phosphorylation in the presence of donor serum was taken as 100% and the value of phosphorylation in the presence of myasthenic serum was compared with it. Such a method ensures greater reliability of results than statistic comparison of experimental data owing to the extensive fluctuation of the rate of phosphorylation in different experiments.

It was found that in the majority of cases myasthenic sera exerted a greater depressing influence on phosphocreatine formation than normal sera. This was found in 10 of the 12 myasthenic patients investigated. Most

of the patients were subjected to repeated examinations; a total of 29 experiments was carried out, and increased depression of phosphorylation was found in 24 (Table 2). The average value for phosphocreatine formation in the presence of serum from myasthenic patients as compared with serum from healthy subjects was 87%.

TABLE 2

Phosphocreatine Formation in the Presence of Serum from Myasthenic Patient (in percentage ratio to values obtained with normal serum taken as 100%)

Name	Before administration of proserine	After administration of proserine
1. Serum from myasthenic patient		
L-va	77	101
T-an	88	103
P-uk	92	110
Z-va	80	89
2. Boiled serum from myasthenic patient		
L-va	71	110
D-ets	83	110
M-di	94	106
T-an	89	—
3. Dialyzate of serum from myasthenic patient		
F-va	82	—
T-an	92	—

To assess the significance of the discovered phenomenon of increased depression of phosphocreatine formation under the influence of myasthenic serum it appeared to be of interest to determine the change, if any, in the properties of the serum in relation to fluctuations in the patients' condition before and after administration of proserine. Sera samples were therefore taken before proserine and 45 minutes after intramuscular injection of 2 ml 0.05% proserine when the condition of the patients was considerably improved. It was found that under these conditions the rate of phosphorylation reached the control values and sometimes even exceeded them (see Table 2).

The greatest change towards enhanced phosphorylation after administration of proserine was observed in severely ill patients. In their case the increase of phosphorylation reached 20-30% as compared to the values observed prior to proserine injection. Control investigation of the effect of proserine itself or of proserine mixed with serum on the processes of phosphorylation revealed that these factors not only exerted no stimulating influence on phosphocreatine formation on direct action on the test substrate in vitro, but even suppressed this process.

The change in the properties of the serum in patients given proserine provides supplementary indication of the fact that the greater depression of phosphocreatine formation by myasthenic serum reflects functionally important features pertaining to myasthenia. Improvement in the functional state of the patient and liquidation of myasthenic disorders are accompanied by changes for the better in the ability of the myasthenic serum to influence phosphocreatine formation.

The distinctive features of the effect of myasthenic sera on phosphorylation are preserved on boiling the serum and on subjecting it to dialysis. Preservation of these properties despite boiling and dialysis permits the conclusion that the factor responsible for the enhanced depression of phosphorylation is, most probably nonprotein in nature, of low molecular weight and does not belong to the enzyme group of compounds. This factor cannot be glucose either, since addition of glucose to the samples in which phosphocreatine is being formed in the presence of serum from a healthy subject does not lead to increased inhibition. Table 2 presents data of some of the experiments on the formation of phosphocreatine under the influence of myasthenic sera before and after administration of proserine as well as of sera after boiling and of serum dialysate (phosphocreatine formation is given as percentage of the values obtained in control experiments taken, in each given experiment, as 100%).

In connection with the discovered phenomenon of phosphocreatine formation inhibition by serum from patients with myasthenia in a state of myasthenic cachexia, a parallel series of investigations was staged (in collaboration with Ya. K. Smirnov) on the effect of myasthenic sera on processes of phosphocreatine formation and tissue respiration in the same sample. In these experiments (20 observations) no parallelism was found between the effect of myasthenic serum on phosphocreatine formation and on tissue respiration. Myasthenic serum, which exerted an inhibitory influence on the formation of phosphocreatine, did not affect tissue respiration perceptibly under the given experimental conditions. These investigations are being pursued further.

SUMMARY

It was demonstrated that the serum of patients with myasthenia depressed the process of phosphocreatine formation in small pieces of rat's muscle to a greater degree than the serum of a healthy person. Following administration of proserine, when there is a marked clinical improvement in the general condition of the patient, this depressant effect of myasthenic serum is reduced. The factor which depresses the formation of phosphocreatine in myasthenia is preserved after boiling and dialysis.

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PHARMACOLOGY

THE EFFECT OF EMBIKHINE* ON THE COURSE OF EXPERIMENTALLY INDUCED FEVER

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The use of Embikhine as a therapeutic agent in lymphogranulomatosis has shown that Embikhine, as well as acting specifically on the infected ganglia, quickly relieves fever, sweating and itching (sometimes after only 1-2 injections) [3]. The action mechanism of Embikhine has not been studied sufficiently. There are data indicating that the chlorethylamines, even in small doses, disturb the nucleoprotein (especially the nucleic) metabolism. To what extent thermoregulation and other aspects of metabolism are affected by the action of chlorethylamines is not clear from the literature.

We investigated the effect of Embikhine on the development and course of an experimental fever reaction (temperature, gas metabolism – oxygen consumption), using small and medium therapeutic doses of Embikhine (0.3-0.5 ml/kg) and a "shock" dose (1 ml/kg) injected intravenously into the auricular vein.

EXPERIMENTAL METHODS

The experiments were done on rabbits. The fever reaction was induced by Vasilyev's method [1] by intravenously injecting an autoclave-processed bouillon culture of B. mesentericus in a dose of 2 ml/kg. The temperature (rectal) was taken every half hour with a medical maximum thermometer. The rabbits' oxygen intake was determined hourly for 15 minutes in a closed system according to Prof. P. N. Veselkin's modification [2] of Regnault and Reiset's method.

A total of 50 experiments were done on 12 rabbits. The experiments were divided into two groups as follows: 1) experiments on intact (non-feverish) rabbits to examine the effect of Embikhine on change in temperature and oxygen intake; 2) experiments in which the effect of Embikhine on the development and course of fever was studied (with Embikhine injected before the fever reaction had been induced and at different stages during the development of the fever).

EXPERIMENTAL RESULTS

The rabbits' normal daily oxygen intake fluctuated slightly (8%) and, according to our data, was an average 10.9 ml/kg per minute. Embikhine injected once in a dose of 0.3 and 0.5 ml/kg into the intact rabbits caused the body temperature to rise slightly (by up to + 0.8°) and the level of oxygen intake to decrease (by an average of 11%), as did repeated injections of Embikhine (injected for three days and on the day of the experiment).

No decrease in temperature was noticed with one injection of Embikhine in the "shock" dose (1 ml/kg) either (the temperature either remained the same or rose 0.2-0.3°); during the first three hours, the oxygen intake decreased slightly, but returned to the original level five hours after the preparation had been injected.

Having thereby established that Embikhine alone did not lower the body temperature in the control (non-feverish) rabbits, we then tested its effect on rabbits with experimentally induced fever.

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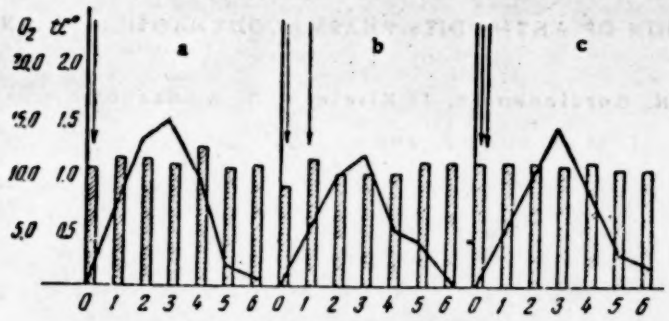
* Original Russian pagination. See C.B. Translation.

When comparing the experimental results from this series of experiments, we established:

1. One injection of Embikhine (0.3 and 0.5 ml/kg) given an hour after the pyrogenic injection had no evident effect on either the rate or amount of temperature rise, nor on the character of changes in oxygen intake, although oxygen intake decreased slightly with the 0.5 ml/kg dose of Embikhine.

2. When a "shock" dose of Embikhine was injected simultaneously with the pyrogenic agent, the development and course of the fever reaction was not noticeably affected.



Effect of Embikhine on the course of an experimentally induced fever reaction. (Rabbit 11).
Columns – changes in oxygen intake; curves – changes in temperature.
a) Control fever reaction, arrow shows the culture injection; b) fever reaction with Embikhine injection (0.5 ml/kg intravenously) one hour after the injection of the B. mesentericus culture; c) fever reaction with simultaneous injection of B. mesentericus culture and Embikhine (1 ml/kg).

3. The maximum temperature of rabbits injected with a "shock" dose of Embikhine an hour after the injection of the pyrogenic agent was 0.2-0.5° lower than that of the rabbits injected with the pyrogenic agent alone; the oxygen intake level was also somewhat lower. We noted that the amount of fluctuation in oxygen intake with fever was somewhat lessened after the Embikhine injection (see Figure).

We therefore propose that the mechanism of Embikhine's antipyretic effect in the treatment of lymphogranulomatosis patients evidently consists primarily of specific action on the pathological process, since Embikhine has no pronounced antipyretic effect.

SUMMARY

The effect of Embikhine on the body temperature and on the changes of the oxygen intake was studied during various stages of experimentally induced fever. It was established that the introduction of Embikhine to the intact animals does not result in decrease of body temperature. Its effect on the rise of temperature or the character of changes of oxygen intake is immaterial (there is only a certain monotony and fewer variations in the intake of oxygen). The mechanism of the antipyretic effect of Embikhine in treatment of patients with lymphogranuloma evidently consists in the specific effect on the pathological process, since Embikhine by itself has no pronounced antipyretic action.

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THE EFFECT OF ANTIGEN ON THE CAROTID SINUS RECEPTORS WITH REFLEX PRODUCTION OF ANTIBODIES PHARMACODYNAMICALLY ANALYZED

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In a previous study, we proposed a new method of isolating the carotid sinus. This method tests the completeness of isolation by the injection of radioactive phosphorus in large doses. The experiments conducted showed that the method we proposed guarantees complete isolation of the carotid sinus, so that radioactive phosphorus injected into the sinus does not appear in the blood. Having developed this method, we attempted once more to verify the possibility of the reflex production of antibodies; we showed that the injection of antigens into an isolated carotid sinus caused, as a rule, the production of antibodies. Antibodies were produced to the antigens injected into the carotid sinus, but did not form to antigens closely related to the antigen injected.

In the same investigation, we established that preliminary sectioning or ligation of the sinus nerve considerably reduces the production of antibodies, sometimes even eliminating it.

In this investigation, we decided to trace the production of antibodies by injecting an antigen previously treated with various substances into an isolated carotid sinus.

EXPERIMENTAL METHODS AND RESULTS

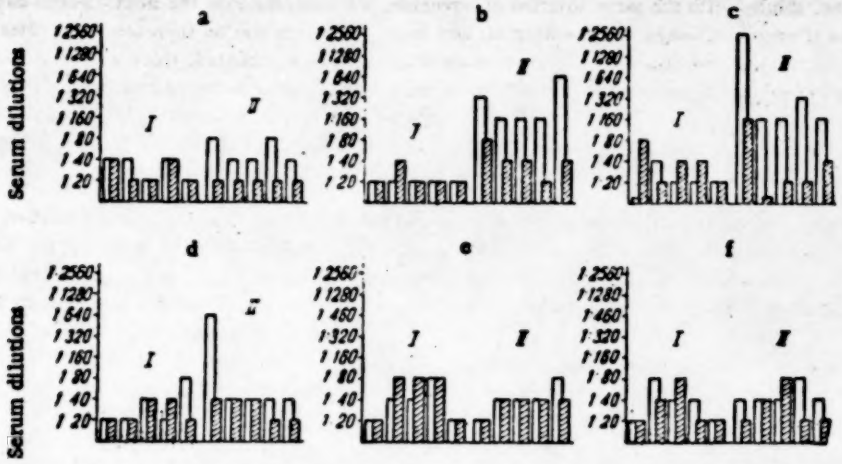
In the first series of experiments, the isolated carotid sinus was preliminarily treated with a physiological solution, and no antigens were subsequently injected. This experimental series showed the effect of operative intervention and of mechanically stimulating the carotid reflexogenic zone on the titer of antibodies present in the blood of each animal. In these and other experiments, we determined the titer of antibodies to the bacilli Bact. typhi abdominalis and Bact. paracoli. The experiments conducted on 5 dogs showed that the titer of antibodies remained practically the same under these conditions.

Therefore, one could affirm that operative intervention alone does not materially affect the titer of antibodies, which remains constant.

In further experiments, also on 5 dogs, we studied the effect of the preliminary action of sodium fluoride on the production of antibodies with the antigen injected into the isolated carotid sinus. The carotid sinus was isolated by our method and then treated with 5% sodium fluoride. Thirty minutes after repeated treatment of the carotid sinus with sodium fluoride, we injected the typhoid vaccine into the sinus. Seven days later, the titer of antibodies to the typhoid bacillus and to the paracolitis bacillus was determined. The experiments showed that the titer of antibodies to the typhoid bacillus had increased rather considerably. If the original titer of antibodies to the typhoid bacillus did not exceed a dilution of 1:20, then immunization increased the titer in two animals to 1:160, in one animal to a dilution of 1:320, and in the other two animals, to a dilution of 1:640; the titer to the paracolitis bacillus did not change in several cases, although it increased by one dilution in two cases.

These data suggest that preliminary treatment of the carotid sinus with sodium fluoride has no effect on the production of antibodies and, probably, that the action of the antigen on the receptors is not realized through change in the carbohydrate metabolism.

In a third series of experiments on 5 dogs, we studied the effect of Novocain on the reflex production of antibodies. The isolated carotid sinus was first treated with a 3% solution of Novocain, which, like the sodium fluoride, was injected into the sinus, where it remained for 20 minutes. After this, the Novocain was extracted and replaced by an injection of the typhoid bacillus. Subsequent determination of the titer 7 days later showed that preliminary treatment of the sinus with Novocain does not check the production of antibodies to the typhoid bacillus; in three animals, the titer of antibodies increased to dilutions of 1 : 160, in one animal it increased to a dilution of 1 : 320 and in the last animal it reached a dilution of 1 : 2560. There was no material change in the titer of antibodies to the paracolitis bacillus.



Change in the titer of antibodies with the antigen injected into a carotid sinus treated with various pharmacological substances.
a) Physiological solution, b) sodium fluoride, c) Novocain, d) Dicaine,* e) cocaine, f) Sovcaine;* I) original titer; II) after 7 days; white columns - Bact. typhi abdominalis; striped columns - Bact. paracoli.

The results we obtained showed that the preliminary injection of Novocain does not materially affect the reflex production of antibodies. This is explained by the fact that Novocain in the concentration used has not the ability to cause terminal anesthesia and therefore, when injected into the sinus, did not materially affect the subsequent action of the antigen on the receptors.

Having established this, we decided to use substances causing surface analgesia. Among these are cocaine, Dicaine and Sovcaine.

In one series of experiments, we isolated the carotid sinus in the animals and then injected a 3% solution of Dicaine into it. Fifteen minutes later, the Dicaine was withdrawn, and the typhoid bacillus vaccine, diluted with Dicaine, was injected into the sinus. The results showed that the titer of antibodies increased to a dilution of 1 : 640 in only one animal, remaining the same in two animals, while in the fourth animal, the titer was reduced, and, in the last case, it increased by one dilution. The titer of antibodies to the paracolitis bacillus was almost unchanged.

This data indicates that, in an absolute majority of cases, preliminary treatment of the sinus with Dicaine checked the subsequent antibody production in the majority of animals. The increase in the titer of antibodies which occurred with one animal we believe to be due to the fact that the carotid sinus receptors were insufficiently anesthetized.

* Russian trade name.

We studied the effect of anesthetizing the carotid sinus receptors with cocaine on 5 animals. In these cases, the sinus was isolated by the method described above, and then 5% cocaine was injected into its cavity. Twenty minutes later, the cocaine was withdrawn, and the typhoid vaccine, diluted with a solution of cocaine, was injected into the sinus. In 4 of the 5 animals of this experimental series, the titer of antibodies in the blood was completely unchanged, and in the other animal it increased to a dilution of 1 : 80 (the titer of antibodies to the paracolitis bacillus also increased in this animal). Therefore, cocaine, like Dicaine, in the majority of cases checks the production of antibodies caused by the action of antigens on a carotid sinus anesthetized by these two substances. Cocaine is known to cause blood vessels to constrict and, therefore, causes the capillaries along the nerve to contract when injected into the carotid sinus.

In order to exclude this possibility, we used Sovcaine in the next experimental series, as it does not cause capillaries to contract, but rather has the ability to dilate them. In this series, as in the others, we isolated the carotid sinus and then injected 5% Sovcaine into it; 15-20 minutes later, the Sovcaine was withdrawn, and the typhoid vaccine, diluted with the same solution of Sovcaine, was injected into the sinus. Seven days later, we determined the titer of antibodies in these animals and found that there was no increase in the titer of antibodies, except for an increase of one dilution in some animals which can be discounted, since a similar change had also been observed in the control experiments. Nor did the titer of antibodies to the paracolitis bacillus change. Therefore, the carotid sinus receptors lose their sensibility when preliminarily treated with Sovcaine and do not react to antigenic stimulation. The result of this is that the antigen injected into the isolated carotid sinus does not cause the titer of antibodies to rise.

All our experiments show that operative intervention and the action of a physiologic solution on the receptors of an isolated carotid sinus do not materially change the titer of antibodies, although the titer did increase by one dilution in some animals. Nor does treating the sinus receptors with sodium fluoride affect the reflex production of antibodies. The titer of antibodies increased in these animals to the same extent as in the animals not preliminarily treated. The action of Novocain on the carotid sinus receptors has little effect on the reflex production of antibodies; the receptors treated with Novocain remain sensitive to antigenic stimulation, and the action of the antigen causes increased production of antibodies. This is explained by the fact that Novocain does not cause terminal anesthesia in the concentration we used.

A completely different picture was observed when the receptors of an isolated carotid sinus were treated with Dicaine, cocaine and Sovcaine – substances which, in the concentrations we used, could definitely cause terminal anesthesia. The receptors treated with these substances lost their sentivity to antigens. The subsequent injection of the antigen into the isolated carotid sinus did not cause the titer of antibodies to increase (see Figure).

The data obtained further convince us that the action of antigens on the carotid sinus receptors causes the reflex production of antibodies.

All this further confirms the part of a reflex mechanism in the production of antibodies from the carotid sinus receptors.

SUMMARY

An operation of isolation of the carotid sinus with the following introduction of the physiologic saline into the carotid sinus has almost no effect on the titer of antibodies in the blood.

Preliminary treatment of receptors of carotid sinus by Novocain and by sodium fluoride does not change its sensitivity to the antigenic stimulation. Introduction of antigen on this background brings about production of antibodies, the titer of which reaches the same level as in antigenic stimulation without preliminary treatment by these substances.

Treatment of receptors of the isolated carotid sinus by cocaine, Dicaine and Sovcaine causes disappearance of the excitability of receptors to the antigenic stimulation. Introduction of typhoid vaccine into the carotid sinus which had been treated by cocaine, Dicaine and Sovcaine does not induce antibody production. The latter supports the view of the reflex mechanism of production of these antibodies.

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* Original Russian pagination. See C.B. Translation.

ONCOLOGY

ON THE ANTITUMOR ACTIVITY OF CERTAIN DERIVATIVES OF SARCOLYSIN (DL-n-di-(2-CHLORETHYL) AMINOPHENYLALANINE)

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Among the synthetic di-2-chlorethylamines sarcolysin [DL-n-di-(2-chlorethyl)aminophenylalanine] has shown in experiments the strongest and widest antitumor activity [1, 2].

At present sarcolysin is employed clinically and has given positive results in treatment of certain malignant tumors and metastases [3].

However, effectiveness of the sarcolysin is limited only to certain human tumors so that there are valid reasons for working on the synthesis of new compounds for the treatment of other tumors.

With this goal in mind, we began a search for other antitumor compounds by modifying the sarcolysin formula, substituting either the carboxyl or amino groups. In addition to the practical bearing, the study of such compounds presents a great theoretical interest as it makes clear the meaning of the presence of the free carboxyl and amino radicals in antitumor sarcolysin activity.

With the above mentioned considerations in mind, we synthesized: 1) two compounds with substitution in the carboxyl group, ethyl and isopropylethers of DL-sarcolysin and 2) two compounds with substitution in the amino group, – DL-N-formylsarcolysin and DL-N-acetylsarcolysin.

EXPERIMENTAL METHODS

The ethylated ether of DL-sarcolysin was obtained by boiling a solution of sarcolysin for six hours in absolute ethanol saturated with dry HCl. This formed the dichlorhydrate ethyl ether of sarcolysin (melting point 174-177°), which hydrolyzed readily, becoming the monochlorhydrate with a melting point of 156-157° (in alcohol).

By an analogous procedure we obtained the monochlorhydrate isopropyl ether of DL-sarcolysin with a melting point of 187-189° (in alcohol).

In order to obtain DL-N-formylsarcolysin, the basic solution of sarcolysin in 96% formic acid was heated and acetic anhydride added drop by drop. The substance obtained had a melting point of 151-152° (in alcohol). At the same time, this compound was obtained by I. L. Knunyants, O. V. Kildisheva and N. E. Golubeva [4].

DL-N-acetylsarcolysin was synthesized by the action of acetic anhydride on sarcolysin dissolved in glacial acetic acid and then heated. The melting point is 152-153° (in alcohol).

EXPERIMENTAL RESULTS

Study of Biological Effects

The toxicity of the preparations was studied in adult rats by means of single intra-abdominal injections. Simultaneously, as standard controls, the toxicity of DL-sarcolysin was also determined.

The minimum lethal doses (LD_{50}) were determined for the preparations, these being the quantity causing the death of 50% of the animals. The results are given in Table 1.

TABLE 1

Dosages of the Preparations Producing Death in 50% of the Animals

Preparation	Dose in mg/kg
DL-sarcolysin	22
Ethyl ether of sarcolysin	17
Isopropyl ether	17
DL-N-formylsarcolysin	130
DL-N-acetylsarcolysin	90

When the animals were autopsied after the single injection had led to their deaths, regardless of the preparation used, there was observed macroscopically a shrinking of the spleen, lymph glands and the thymus, intestinal distension with fluid content and other signs characteristic of the action of the chlorethylamines.

Antitumor Activity

The antitumor activity of the preparations was studied on rats inoculated by the usual method with spindle cell sarcoma-45, done in the laboratory of experimental chemotherapy of the Institute of Experimental Pathology and Cancer Therapy. The antitumor activity of the preparations was compared with that of DL-sarcolysin.

There were three experimental modifications: 1) daily injections for 20 days; 2) a total of 7 injections at 72 hour intervals; 3) a single injection only.

In all instances, therapy commenced on the 7th day following tumor inoculation; animals were sacrificed on the 21st day after the beginning of therapy (on the 28th day following inoculation). Every 5 days the tumors were measured in three different directions and the average of the diameters was taken; its alterations being taken as an index of the dynamics of the preparation.

After being sacrificed, the animals were autopsied and the tumors stripped out, weighed and the percentage of growth inhibition determined.* In addition, the percentage of tumor resolution was determined separately. As was shown previously [1, 2], when sarcolysin dissolves sarcoma-45, there remains either a pigmented spot or a small soft node at the site of the former tumor. Histological examination has established that these nodes contain no tumor cells and disappear later after therapy has been terminated. We considered the tumor to have dissolved when such a nodule weighed 0.05 g or less. Nodes weighing over 0.05 g we counted as tumor tissue and we took their weight into account when determining the average weight of the tumors. Altogether we ran 4 series on 229 rats.

The results of the experiments are shown in Table 2.

As can be seen from Table 2, all four of the preparations studied have definite antitumor activity. So we have shown that antitumor activity is present both when the carboxyl group is substituted and also when the amino group of the phenylalanine in the sarcolysin molecule is substituted.

However, a comparison shows many biological differences in the actions of the preparations.

* Percentage of inhibition was calculated as usual from the following formula:

$$\frac{\text{average tumor weight in control} - \text{average weight in treated animals}}{\text{Average tumor weight in control animals}}$$

TABLE 2

Influence of Sarcolysin Derivatives Upon Tumor Growth

Expt. No.	Preparation	Number of animals: controls	Number of animals: expti.	Number of animals: dying during expt.	Dose in mg/kg	Interval between doses, hrs.	No. times prep'n. was introduced, mg/kg	Quantity of prep'n introduced, mg/kg	Average tumor weight: controls	Average tumor weight: treated animals	% inhibition	% dissolution
10	Sarcolysin	10	10	0	1.2	24	20	24	47.4	4.18	91.3	0
	Ethyl ether	10	10	0	1.2	24	20	24	47.4	8.5	82.0	0
	Isopropyl ether	10	10	0	1.2	24	20	24	47	10.83	77.2	0
11	N – formylsarcolysin	10	10	1	30	24	20	600	50.0	0.84	98.3	40
	"	10	10	0	10	24	20	200	50.0	13.75	61.2	0
13	Sarcolysin	10	10	0	1.5	24	20	30	53.7	4.50	91.6	20
	N – acetylsarcolysin	10	10	0	2.5	24	20	50	53.7	4.17	92.2	10
12	Sarcolysin	10	10	0	5.0	72	7	35	28.7	0.095	99.6	80
	Ethyl ether	10	10	0	5.0	72	7	35	28.7	0.148	99.5	60
	Isopropyl ether	10	10	0	5.0	72	7	35	28.7	0.0	100	100
	N – formylsarcolysin	10	10	1	30.0	72	7	210	28.7	2.45	91.4	20
	N – acetylsarcolysin	10	12	5	20.0	72	7	140	28.7	0.08	99.7	71
13	N – acetylsarcolysin	10	10	0	10.0	72	7	70	53.7	3.0	94.4	10
	Sarcolysin	10	10	1	6.0	72	7	42	53.7	0.0	100	100
12	"	10	10	0	15	Only once		15	28.7	0.12	99.5	70
	Ethyl ether	10	10	4	15			15	28.7	0.23	99.1	33
	Isopropyl ether	10	10	1	15			15	28.7	1.18	96.0	33
	N – formylsarcolysin	10	10	0	60			60	28.7	16.22	47.2	10
	N – acetylsarcolysin	10	10	0	20			20	28.7	0.04	99.8	70
13	N – acetylsarcolysin	10	9	0	30			30	53.7	0.75	98.6	66

When the carboxyl group is replaced by ethyl or isopropyl ether, both compounds resemble each other in their toxic and antitumor actions. Both the preparations are somewhat more toxic than sarcolysin and approach it in antitumor activity. When the amino group is substituted, the toxicity of the preparations drops markedly. Thus, for DL-N-formylsarcolysin the LD_{50} increases by a factor of 6 while for DL-N-acetylsarcolysin the toxicity factor rises fourfold when compared with DL-sarcolysin. The preparations differ from each other in their antitumor activity.

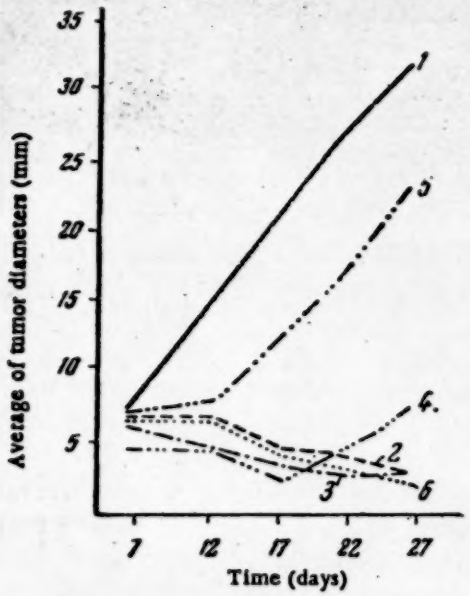

Fig. 1. Changes in the average diameters of the tumors, sarcoma-45, following a single injection of DL-sarcolysin (2), ethyl ether DL-sarcolysin (3), isopropyl ether DL-sarcolysin (4), DL-N-formylsarcolysin (5), and DL-N-acetyl-sarcolysin (6) all compared with the control curve (1).

The daily injection of DL-formylsarcolysin, whose dosage is 8 times that of sarcolysin, still gives a much weaker effect. In order to obtain a result approaching that obtained with sarcolysin, the dose has to be 25 times that of sarcolysin to produce death in some of the animals. The same occurs with other experimental modifications. Thus, in this preparation, the disparity between the toxic and therapeutic dosages is less than seen in sarcolysin and so this compound can have no practical value even when offering much theoretical interest.

On the other hand, DL-N-acetylsarcolysin produces an effect corresponding to that of sarcolysin in all experimental modifications, the dose exceeding that of sarcolysin only $1^1/_2$-2 fold. Thus, this preparation has a much greater disparity between the toxic and therapeutic dosage than seen with sarcolysin.

Eight rats, in whom therapy with DL-acetylsarcolysin led to total tumor resorption, are still under continuing observation 2 months later. In this time no recurrences have been observed. This may mean that this compound will have practical value.

Final judgment as to the clinical applicability of the preparations will have to await a study of their effects on blood and animal organs, as well as their spectrum of antitumor activity.

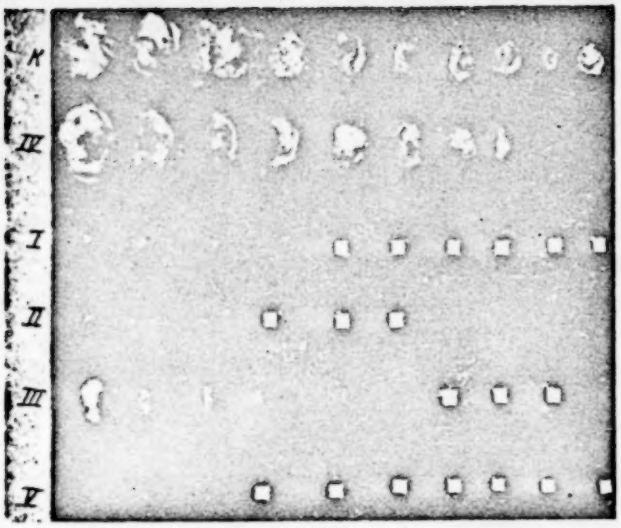

Fig. 2. Tumors (sarcoma-45) on the 21st day after a single injection of the preparations in the same dosages.
k-control. I) DL-sarcolysin; II) ethyl ether DL-sarcolysin; III) isopropyl ether DL-sarcolysin; IV) DL-N-formylsarcolysin; V) DL-N-acetylsarcolysin. White squares indicate rats in whom the tumors were fully resorbed.

The comparison of the toxic and antitumor activities of DL-N-formyl- and DL-N-acetylsarcolysin preparations leads us to take a great interest in examining replacement of the amino group on the sarcolysin with a longer alkylated chain. These studies are now in progress.

SUMMARY

Four modifications of sarcolysin were prepared. Two were substitutions in the carboxyl group: ethyl and isopropyl ethers of DL-sarcolysin; and two were substitutions in the amino group: DL-N-formylsarcolysin and DL-N-acetylsarcolysin. Animal tests were performed on rat sarcoma 45, a total of 229 rats being used to determine the LD_{50} and also the antitumor effect.

The carboxyl group substitutions were very similar in effects to sarcolysin itself. The amino substituted compounds were both less toxic and less effective against tumors. However, while in N-formylsarcolysin the difference between the toxic and therapeutic doses is less than in sarcolysin, the lethal dose of the N-acetylsarcolysin is about 4 times greater than the dose for sarcolysin itself. Thus, the therapeutic dose of this last modification has twice the safety of the corresponding sarcolysin dosage.

This last result has aroused interest in replacing the amino group with longer alkylated chains. Studies with such compounds are now in progress.

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RESORPTION OF UNTREATED METASTASES AFTER SUBJECTION OF RABBIT BROWN-PEARCE TUMORS TO HIGH-INTENSITY ULTRASONIC WAVES

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In communications from our laboratory we have reported that Brown-Pearce tumors in rabbits were made to resorb after being subjected to high-intensity ultrasonic waves [1]. Histological examination of the tumors immediately following their treatment with ultrasonics did not indicate any evidence of morphological alterations. However, the survival capacity of the tumor cells is markedly diminished. This is manifested by the sharp decrease in the multiplication of the tumor cells and the development of dystrophic processes in the tumor tissue. This progressive dystrophy of the tumor cells leads to their total regression. The tumor either becomes resorbed tracelessly or is replaced by scar, or else loses all distinguishing features and becomes encapsulated.

The examination of the untreated metastases and the study of the changes going on in them while the primary tumor which had been subjected to ultrasonic waves is undergoing regression, presents a topic of the greatest interest. This communication is devoted to an examination of this subject.

In our experiments we used transplants of the Brown-Pearce tumor which is characterized by intensive and swift metastases, so that castration 1 to 6 hours after introduction of the tumor material does not prevent metastatic spread [3, 4]. When we used the classical procedure of inoculating the tumor material into the testicle, we found ourselves in agreement with other workers in stating that in only 6 to 8% of the total could spontaneous tumor regression be observed. However, the method of inoculation used is important in determining the frequency of metastases to the inner organs and the percentage of spontaneously regressing tumors. Thus, when the tumor material was introduced intramuscularly, in our experiments 27.3% of the cases showed spontaneous resorption of the swellings.

EXPERIMENTAL METHODS

Our experimental group included 23 rabbits: a control group – 12 which had manifested spontaneous resorption of the primary tumor nodule, isolated from 112 rabbits which had been injected intramuscularly and intratesticularly with Brown-Pearce material; and an experimental group of 11 having both tumor and metastases which were observed for indications of tumor recession after various regimens of ultrasonic treatments.

The Brown-Pearce tumor was given to both the control and further experimental group of rabbits intramuscularly and intratesticularly. The tumor tissue was pulverized, suspended in saline solution (1:3) and injected in the amount of 0.8 to 1 cc. To facilitate the application of ultrasonic waves, the testicle was displaced under the skin on the forward surface of the thigh and fixed in place with a stitch. A similar operation was performed on the control rabbits. The animals received ultrasonic treatments from the powerful installation built by A. K. Burov [2]. The treatment of the tumor nodules was conducted after the method of G. D. Andreevskaya on the 7 to 11th day after the tumor inoculation, i. e., at a time when metastasis has occurred and when macroscopic evidence of numerous metastatic nodules can be seen on autopsy.

The method of application was as follows: contact was established through vaseline and with sound waves conducted through the liquid medium of water. The treatment regimen: frequency of 750-1500 kilocycles; power of 27-150 W/cm^2; exposure 1.3-75 seconds.

The experimental and control groups were examined carefully each week. The animals which died or were sacrificed at the end of the experiment were examined thoroughly on autopsy. The rabbits were observed from a period of several months (3 to 4) up to $3^1/_2$ years.

EXPERIMENTAL RESULTS

In our studies we observed spontaneous regression of the primary tumor only in those rabbits which were still free of metastases. In these animals we were never able to see or palpate metastases. This absence of metastasis was also confirmed by subsequent autopsies as was the absence of any scars or calcifications within the internal organs and lymph nodes. We also failed to uncover any reports in the literature of the spontaneous regression of a primary growth due to Brown-Pearce tumor and metastases.

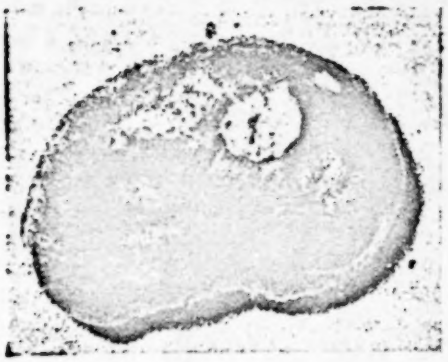

Fig. 1. Foci of dedifferentiation and scarring on the site of former metastases in rabbit No. 117/717 $3^1/_2$ years after treatment with ultrasonic waves. Macropreparation.

Our observations have shown that when the Brown-Pearce tumor regresses spontaneously, the tumor nodule gradually diminishes in size until it is totally gone at the end of 1-2 months following the inoculation. The action of ultrasonic waves leads not only to resorption of the nodule subjected to treatment but also, in many instances, to resorption of untreated metastases. In the experimental rabbits the regression of the treated nodule was usually preceded by some growth of the tumor nodule. The regression process of the swelling in the various animals took from 15 days to 5 months. Naturally, we fixed our attention upon the metastases forming in the eyes, lymph glands, and kidneys as we could observe them visually and with the aid of palpation. In addition, the exact condition of the metastases was defined at autopsy and studied further histologically.

Our observations demonstrated that before the treated tumor begins to regress there is some enlargement of the metastatic nodes. Later, these sharply diminish in size and disintegrate. The disintegrated metastases, as was the case with the primary nodule, subjected to ultrasonic waves either became replaced by scar tissue, became dedifferentiated and encapsulated, or else vanished without leaving any trace. The process of metastatic disintegration lasts a long time and ends only long after the primary nodule has disappeared completely. Thus, in some instances it took 7 months for the metastases in the lymph glands, eyes and kidneys to dissolve.

We present our data in the following concrete observations.

Instance 1. Regimen of treatment: frequency 750 kilocycles, power 34 W/cm^2, exposure 15 seconds repeated five times. The contact was aided with vaseline.

In rabbit No. 780 the tumor was inoculated into the muscle of the right thigh. Eleven days later the tumor nodule was subjected to ultrasonic waves. Within $2^1/_2$ months the tumor had dissolved. By that time the lymphatic gland under the knee could be palpated as being thickened. Nine months later the rabbit was sacrificed and autopsied, and a scar was found at the site of the primary nodule. The lymphatic gland under the right knee and the right adrenal were walnut sized, thickened and surrounded by a dense capsule which when sectioned had the appearance of a reddish caseated mass. Microscopic section revealed dystrophic tumor tissue. Fields of complete lysis of tumor elements alternated with areas consisting of small rounded cells with wrinkled, dark eccentrically placed nuclei. In the lungs there were also found large disintegrated metastatic nodes, the size of walnuts, and smaller ones no larger than small peas. In these latter, histological studies showed cellular disorganization, vacuolization and pyknosis of the nuclei, karyorrhexis.

Thus, in this rabbit, after the disintegration of the treated primary nodule, resorption of metastases took place in the lungs, adrenals and lymph gland below the knee. The ultrasonic regimen as outlined produced similar regression of metastases in 3 more rabbits: Nos. 559, 749 and 781.

Instance 2. Regimen of treatment: frequency 1500 kilocycles, power 27 W/cm^2, exposure 17 seconds. Ultrasonic waves were conducted through water.

Rabbit No. 117/717 was inoculated intratesticularly with the tumor, the testicle having been placed under the skin of the right thigh. Eight days later, the rabbit was subjected to treatment. On that day the tumor measured 5 x 1 x 1.5 cm. It continued to enlarge for 2 months after the application of ultrasonic waves. Its dimensions reached 7.5 x 4 x 2 cm. By this time the rabbit had metastases in the right eye and in the kidneys. Starting at 2 months after the treatment, the tumor began to decrease and was fully resorbed after 5 months. The metastasis observed in the right eye became necrotized. After 3 months the disintegrated tumor in the eye began to separate from the eye ball. After 4 months the orbital depression was clear of the necrotic mass and the rabbit began to move the eye-lids freely. The metastases in the kidneys were palpable as hillocks for almost 6 months. Then they began to decrease in size and could not be palpated at 7 months. The rabbit was sacrificed 3$^1/_2$ years after the ultrasonic treatment. At autopsy both kidneys were seen to have dense rounded foci of dedifferentiation of about 5-8 mm diameter. (Fig. 1). Histological examination confirmed the dedifferentiation and scar transformation of the tumor mass (Fig. 2).

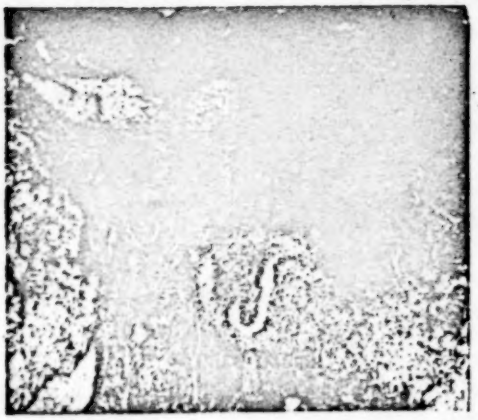

Fig. 2. Scar at the site of former metastases in the kidney (same rabbit as in Fig. 1). Section stained with picrofuchsin (enlargement 10 x 10).

Thus, in this instance, following regression of the treated tumor the metastases in the eye resorbed after 4 months and in the kidneys after 7 months.

In rabbits, No. 17 and 10/611, subjected to the same treatment, regression of the primary tumor inoculated intratesticularly was observed, this being followed by resorption of the tumor conglomerates in the seminal canal, measuring 4 x 1.5 cm, which had not been treated.

In subsequent experiments we tripled the intensity of the ultrasonic waves and correspondingly shortened the exposure time. In the treated rabbits we observed complete cessation of the tumor process as described below.

Instance 3. Regimen of treatment: frequency 1500 kilocycles, power 87 W/cm^2, exposure 3 seconds. The ultrasonic waves were conducted through water.

In rabbit No. 708 the tumor was inoculated into the testicle displaced forward under the skin. Seven days following the inoculation, the tumor, measuring 3.5 x 2.5 x 1 cm was subjected to treatment. For the first 10 days following the treatment the tumor continued its growth attaining 5 x 3 x 1.5 cm. Then the tumor nodule began to soften and decrease in size. 1$^1/_2$ months after the procedure the swelling had fully resorbed. 4 months later the rabbit was sacrificed and at autopsy disintegrated petrified metastases were found in the right lymph gland and adrenal.

In rabbit No. 707 from the same experiment the tumor grew at first markedly after the experimental treatment but then began to regress and became encapsulated, remaining about walnut sized. 17 days after the procedure there appeared metastases in the left eye and in the kidneys where they could be felt on palpation as hillocks. 3 months later the kidney metastases could no longer be felt. The rabbit died 5$^1/_2$ months from the day of inoculation as a result of a traumatic paralysis of the posterior extremities. Histological investigation of the treated tumor nodule showed a picture of total necrosis of the cancer tissue and formation of focal dedifferentiation. Both kidneys appeared normal in size. On their surfaces, where previously the metastases could be palpated, there were numerous depressions of varying size with a whitish, convex bottom. Histological examination of these areas uncovered coarse fibrous scar tissue.

Fig. 3. Organization of a liver metastasis in rabbit No. 117 three weeks after ultrasonic treatment. Section stained with picrofuchsin, enlargement 7x8.

Instance 4. In these experiments the intensity of the ultrasonics was 5 times that used in the first experiments, while the decrease in exposure time was 7-fold. System of treatment: frequency 1500 kilocycles; power 150 W cm^2; exposure 1.3 seconds; ultrasonic conduction through water.

In these experiments also, we observed that along with resorption of the treated primary tumor regression of the untreated metastases took place. Thus, in rabbit No. 80 the tumor dissolved $1\frac{1}{2}$ months after the procedure. By that time, the right eye was observed to have a large metastasis. 5 months later the eye metastasis disintegrated, the necrotic masses sloughing out. Another rabbit, No. 117, was sacrificed 3 weeks after the ultrasonic treatment and a liver metastasis could be seen organizing (Fig. 3).

Summarizing our experiments, we draw the following conclusions:

1. The action of high-intensity ultrasonic waves leads to disintegration of Brown-Pearce tumor nodules in rabbits and, in many instances, leads to disintegration and resorption of untreated metastases in such places as the eyes, lungs, liver, adrenals, lymph glands and so on.

2. Just as with the treated primary tumor nodules, the disintegrated metastases may be replaced by scar tissue, dedifferentiated and encapsulated or tracelessly resorbed.

This must mean that high-intensity ultrasonic waves not only act directly on the primary tumor but also, apparently, produce such alterations in the animal organism that the entire cancerous growth is checked and regresses. This problem must become the subject of special investigations.

SUMMARY

The action of high-intensity ultrasonic waves leads to disintegration of Brown-Pearce tumors inoculated into rabbits a week or more previously. In addition, the untreated metastases have been shown in many instances to undergo similar regression. Just as with the primary, treated nodule, they become replaced by scar tissue, dedifferentiate and encapsulate or resorb tracelessly.

This must mean that the intense ultrasonic waves act not only on the primary tumor directly, but also, must produce basic alterations in the animal organism as a whole which enable it to check the developing cancer everywhere in the body.

This fundamental problem must become the subject of special investigations.

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A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF CERTAIN MOUSE TUMORS INOCULATED INTO PURE-BRED MICE

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The discovery of specific tumorous antigens [1-7] necessitated a more careful study of them. The problem concerning the specificity of the tumorous antigens (especially with transplanted tumors) must be further developed since many works have appeared in foreign literature which throw doubt on the presence of specific immunity in the case of tumors.

Foreign immunogeneticists [8, 9] explain the existence of immurity in transplanted tumors by a tissue incompatibility between the tissues of the animal bearing the tumor and those of the recipient due to their immunogenetic difference.

Th. Hauschka [9] believes that the antigenic conformity of the tumor donor's tissues with those of the recipient does not cause an immune reaction, and that the tumor develops without the appearance of antibodies. This author believes that the antibodies discovered in non-infiltrating tumors are associated with the phenomena of iso- and autoimmunization. Certain foreign scientists have investigated the problem concerning the existence of specific tumorous antigens using inbred animals [10]. They concluded that a specific cancer antigen was present even when a tumor was passed to pure-bred animals of the same genetic breed, a process effecting maximal exclusion of iso-antigenic differences.

In 1956, V. V. Gorodilova and L. V. Shershulskaya conducted experiments using the anaphylactic reaction with desensitization to study the antigenic properties of normal tissues in mice of various breeds. These authors were unable to discover any antigenic difference in normal tissues of genetically different animals breeds.

We attempted to clear up these differences of opinion. For this purpose, we conducted a series of experiments studying the specific tumorous antigens of 3 transplanted mouse tumors, inoculated simultaneously into the same animal and then passed many times to mice of the A. breed. The inoculation of all 3 tumors into one inbred animal and the subsequent passing of the tumors for a long period of time maximally diminished the influence of the genotypic differences present in non-pure-bred animals, and also reduced the iso-antigenic difference existing between individuals of the same breed.

EXPERIMENTAL METHODS

We made a comparative study of 3 mouse tumors: Ehrlich's adenocarcinoma (subcutaneous form), Kroker's sarcoma and acridine sarcoma. The original inoculation was done by means of a trocar, transplanting uniform pieces subcutaneously to white mice of breed A. Ehrlich's adenocarcinoma was inoculated into the left cervical region, Kroker's sarcoma, into the right flank, and acridine sarcoma into the left flank of the animal.

Therefore, all three tumors developed simultaneously on the same mouse. Fifteen to eighteen days later, the tumors were passed to inbred mice of breed A. in the same locations. A total of 20 passages were done.

We examined the tissues of the passed tumors immunologically, using the complement fixation reaction (CFR) both in its usual form and with preliminary specific absorption of the antitumor immune serums according to the method proposed by P. N. Kosyakov [4].

The immune serums were obtained from chinchilla rabbits intravenously immunized according to one of the 4-week cycle systems. Immunization was produced to each of the 3 tumors inoculated into the same animal at the 1st, 10th and 20th passages. We used the complement fixation reaction in the control experiments with immune serums in relation to the normal mouse liver and spleen.

EXPERIMENTAL RESULTS

In the first series of experiments, we studied the changes in the antigenic properties of tumors passed for a long time to inbred mice. We used immune serums in relation to the Ehrlich's adenocarcinoma (No. 1353 and 1393), to the Kroker's sarcoma (No. 1692 and 3243) and to the acridine sarcoma (No. 1094 and 343) of the 1st and 20th passages. Their examination with the suitable antigens in the complement fixation reaction gave the results presented in Table 1.

As the data in Table 1 shows, the complement fixation reaction did not disclose any change in the antigenic properties of the tumorous tissues to occur during the passing process. Each of the antitumor serums obtained to the tumors of the 1st passage reacted with the corresponding antigen in approximately the same titer as with the antigen from the tissue of the same tumor passed 20 times. The antitumor serums to the tumors of the 20th passage also had the same titer with the antigens from the tissues of the tumors passed 20 times as with the antigens from the tissues of 1st passage tumors. Therefore, prolonged passaging of the tumors to pure bred mice did not affect the antigenic properties of these transplanted tumors. By passing the 3 transplanted tumors simultaneously to inbred mice, we obtained data on the similarity and difference in the antigenic properties of the tumors mentioned.

Such an experimental form excluded the influence of individual antigenic differences in the tissues of the mouse tumor host, since all three tumors developed on one body.

We were also interested in finding additional proof that cancer antigens, specific only to tumors, are present in tumorous tissues as well as the normal antigens. We first tried the ordinary complement fixation reaction for this purpose, without the use of specific absorption. Therefore, in the second series of experiments, we used immune serums to each of the tumors of the 20th passage, with immune serums against normal mouse liver and spleen as the control. Table 2 is a report from a typical experiment with the complement fixation reaction.

Table 2 shows that all the antitumor serums reacted in approximately the same titers with their own antigens and with the antigens from the two other tumors, although the titers were somewhat higher in the reaction with their own antigens. The serum against the normal liver (No. 2991), however, reacted considerably more strongly with its own antigen than with the antigens from the tumors.

The same can be said of the antitumor serums in relation to the normal liver antigen, with which they did not react as strongly as with the tumor antigens. The serum against the mouse spleen reacted with both the tumor and spleen antigens in approximately the same titers, which was also true of the antitumor serums in relation to the antigen from the mouse spleen.

Therefore, the ordinary form of the complement fixation reaction showed that the antigens of these three tumors were very similar, that the antigenic properties of the three tumors differed from those of the normal liver and resembled those of the normal spleen.

In order to establish whether there actually were antigenic differences between the tumors and in order to differentiate the antigenic properties of the tumors from those of the normal spleen, we resorted to the method using preliminary specific absorption of antitumor serums. We used both formalized and native tissues from the tumors to absorb the serums. No material difference was found between absorption by the native tumors and that by the formalized material, although the serums were anticomplementary in some cases, when the serums had been insufficiently centrifuged after absorption by native tissues.

TABLE 1

Comparative Study of the Antigenic Properties of Tissues From the Three Transplanted Tumors (Ehrlich's Adenocarcinoma, Kroker's Sarcoma and Acridine Sarcoma) Passed 1-20 Times

Dilution of serums	Antitumor serums against:											
	Ehrlich's adenocarcinoma				Kroker's sarcoma				Acridine sarcoma			
	1st passage, No. 1353		20th passage, No. 1393		1st. passage, No. 1692		20th passage, No. 3243		1st passage, No. 1094		20th passage, No. 343	
	Antigens obtained from the passed tumors											
	from Ehrlich's adenocarcinoma				from Kroker's sarcoma				from acridine sarcoma			
	1st passage	20th passage	1st passage	20th passage	1st passage	20th passage	1st passage	20th passage	1st passage	20th passage	1st passage	20th passage
1:50	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:100	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:200	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	++++
1:400	++++	+++	+++	+++	+++	+++	+++	++++	+++	++	+++	+++
1:800	+++	+++	+++	+++	+++	++	+++	++	+++	+	+++	++
1:1 600	++	++	++	+	++	+	++	++	++	+	++	++
1:3 200	+	+	+	+−	+−	+−	+	+	+	+−	+	+
1:6 400	−	−	+−	−	−	−	+−	−	−	−	−	+−

Note: All necessary controls were observed in the complement fixation reaction.

TABLE 2

Comparative Study of the Antigenic Properties of Tumorous and Normal Tissues, Using The Complement Fixation Reaction

Anti tumor serum	Dilutions	Antigens					Serum controls
		Ehrlich's adeno-carcinoma	Kroker's sarcome	Acridine sarcoma	Mouse liver	Mouse spleen	
No. 198, against Ehrlich's adeno-carcinoma	1:50	++++	++++	++++	+++	++++	–
	1:100	++++	++++	++++	++	++++	–
	1:200	++++	+++	+++	+	+++	–
	1:400	+++	+++	++	–	++	–
	1:800	++	+	+	–	+	–
	1:1 600	+	–	+–	–	–	–
No. 3215, against Kroker's sarcoma	1:50	++++	++++	++++	+++	++++	–
	1:100	++++	++++	++++	++	+++	–
	1:200	++++	++++	++++	+	+++	–
	1:400	+++	+++	+++	+–	++	–
	1:800	+	+++	++	–	+–	–
	1:1 600	+–	+	+–	–	–	–
No. 810, against Acridine sarcome	1:50	++++	++++	++++	+++	++++	–
	1:100	++++	++++	++++	++	+++	–
	1:200	+++	++++	++++	+	+++	–
	1:400	++	+++	+++	–	++	–
	1:800	+	++	+++	–	+	–
	1:1 600	–	+	++	–	–	–
No. 2991, against normal mouse liver	1:50	+++	+++	+++	++++	+++	–
	1:100	++	+	++	++++	++	–
	1:200	+–	+–	+	++++	+	–
	1:400	–	–	+–	+++	+	–
	1:800	–	–	–	+++	+–	–
	1:1 600	–	–	–	+	–	–
No. 12, against normal mouse spleen	1:50	++++	++++	++++	+++	++++	–
	1:100	++++	++++	++++	++	++++	–
	1:200	+++	+++	+++	+	++++	–
	1:400	++	++	+++	+–	+++	–
	1:800	+	+–	++	–	++	–
	1:1 600	+–	–	+	–	++	–
Antigen controls		–	–	–	–	–	–

Table 3 shows the data obtained from the third experimental series using preliminary specific absorption with the complement fixation reaction.

As the data in Table 3 indicate , the preliminary absorption of the antitumor serums by the tumor tissues removed the incidental antibodies from them and left only the antibodies specific for the tumors against which the serums were obtained. For example, before absorption, serum No. 198 against Ehrlich's adenocarcinoma reacted in a titer of 1:400 with all three tumors, showing their antigenic similarity. Serum absorption by the

TABLE 3

Comparative Study of the Antigenic Properties of the Three Transplanted Tumors (20th Passage), Using the Specific Absorption Method in the Complement Fixation Reaction

No. of serum	Absorbed by tissue from:	Dilutions	Antigens obtained from: Ehrlich's adenocarcinoma	Kroker's sarcoma	Acridine sarcoma	Normal spleen	Serum controls
	Before absorption	1:50	++++	++++	++++	++++	−
		1:100	++++	++++	++++	++++	−
		1:200	++++	++++	++++	++++	−
		1:400	++++	++++	+++	+++	−
No. 198, against Ehrlich's adenocarcinoma	Ehrlich's adenocarcinoma	1:50	−	−	−	−	−
		1:100	−	−	−	−	−
		1:200	−	−	−	−	−
		1:400	−	−	−	−	−
	Kroker's sarcoma	1:50	++++	++	++	+	−
		1:100	++++	+	+	+−	−
		1:200	++	−	−	−	−
		1:400	+	−	−	−	−
	Acridine sarcoma	1:50	++++	++	++	++	−
		1:100	+++	+	+−	+−	−
		1:200	++	−	−	−	−
		1:400	+	−	−	−	−

Ehrlich's adenocarcinoma tissues removed all the antibodies present, and the serum gave a negative reaction with all the tumor antigens. With serum absorption by the tissues of the Kroker's and acridine sarcomas, the incidental antibodies were absorbed, leaving the antibodies specific to Ehrlich's adenocarcinoma, with the antigen from which, serum No. 198 reacted in considerably higher titers. We obtained analogous data concerning serums No. 810 (against acridine sarcoma) and No. 3215 (against Kroker's sarcoma). Their absorption by the tumor tissues made it possible to distinguish the antigenic substances of the tumors and spleen from each other. The absorbed antitumor serum reacted with the corresponding tumor antigen, but not with the antigen from the tissues of the normal spleen, thereby confirming their antigenic difference.

Therefore, the use of the preliminary specific absorption method in the complement fixation reaction clearly showed that the experimental tumors differed from each other in this relation, in spite of the mentioned antigenic community between them, as well as from the tissues of normal organs.

TABLE 3 (continued)

No. of serum	Absorbed by tissue from:	Dilutions	Antigens obtained from: Ehrlich's adeno-carcinoma	Kroker's sarcoma	Acridine sarcoma	Normal spleen	Serum controls
	Before absorption	1 : 50	++++	++++	++++	++++	−
		1 : 100	++++	++++	++++	++++	−
		1 : 200	++++	++++	++++	+++	−
		1 : 400	+++	++++	++++	++	−
No. 810, against acridine sarcoma	Acridine sarcoma	1 : 50	+	+	++	+−	−
		1 : 100	+−	−	+	−	−
		1 : 200	−	−	−	−	−
		1 : 400	−	−	−	−	−
	Kroker's sarcoma	1 : 50	+++	+	++++	+	−
		1 : 100	+	−	+++	−	−
		1 : 200	−	−	++	−	−
		1 : 400	−	−	+	−	−
	Ehrlich's adeno-carcinoma	1 : 50	++	++++	++++	++	−
		1 : 100	+	++	+++	+	−
		1 : 200	−	+	++	−	−
		1 : 400	−	+−	++	−	−
	Before absorption	1 : 50	++++	++++	++++	++++	−
		1 : 100	++++	++++	++++	+++	−
		1 : 200	++++	++++	++++	+++	−
		1 : 400	++++	++++	++++	++	−
	Kroker's sarcoma	1 : 50	++	+++	++	+	−
		1 : 100	+−	+	+	+−	−
		1 : 200	−	−	−	−	−
		1 : 400	−	−	−	−	−
No. 3215, against Kroker's sarcoma	Acridine sarcoma	1 : 50	++	++++	++	++	−
		1 : 100	+	+++	+−	+	−
		1 : 200	+−	++	−	−	−
		1 : 400	−	+	−	−	−
	Ehrlich's adenocarcinoma	1 : 50	+++	++++	+++	+++	−
		1 : 100	+	++++	++	−	−
		1 : 200	−	++	−	−	−
		1 : 400	−	+	−	−	−
Antigen controls			−	−	−	−	−

Since the three tumors were passed to the same animal, we can consider that the differences disclosed in the antigenic properties of the tumor tissues are not antigenic differences in the tissues of individual animal tumor-bearers, as all three tumors developed on the same mouse and had been passed to inbred animals for periods of over a year. Moreover, the investigation of the tumors passed 20 times established the presence of a specific antigen, differing from the antigenic substances of normal tissues, as a rule, since, in the first experimental series, we found that the antigenic properties of the tumors of the 1st and 20th passages were the same.

SUMMARY

A comparative study of the antigenic properties of the 3 transplanted mice tumors was conducted. These tumors were passed for a long period of time in inbred animals, with simultaneous inoculation of all of the 3 tumors to the same mouse. The method of specific absorption of antitumor serums was used in the reaction of complement fixation.

A clear antigenic difference of the tumor tissues from the tissues of the normal organs of the mouse, the carrier of the tumor was revealed as a result of this investigation. Besides, it was demonstrated that the antigenic substances of the cancer cells are common in different tumors. An actual difference between the antigenic properties of the 3 mice transplanted tumors was, likewise, revealed.

The use of these methods allows us to show the fallacy of the opinion of certain foreign immunogeneticists on the absence of the specific immunity in tumors.

The above data not only make the presence of the specific antigenic substances of the pathologically changed cancer cell quite certain, but, likewise, the presence of the variations between the antigenic properties of the 3 different transplanted mice tumors.

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EXPERIMENTAL BIOLOGY

SECONDARY DIFFERENTIATION OF EXPLANTED EMBRYONIC HEART MUSCLE

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We know of only one report on the secondary differentiation of explanted mammalian heart muscle, published in 1932 by Goss [2]. N. G. Khlopin [1], in his monograph "Biological and Experimental Principles of Histology," correctly pointed out that Goss's material represented persisting muscle fibers, and not secondary differentiation of explanted heart muscle. Goss published only one photomicrograph of a living, unstained culture of heart muscle, showing numerous differentiated muscle fibers, all oriented strictly in one direction, and firmly and uniformly bound to each other, filling the whole field. There can be no doubt that Goss was dealing with persisting differentiated muscle fibers, and not with secondary differentiation of elements of explanted heart muscle. It may thus be said that secondary differentiation of heart muscle outside the body has not yet been observed, and this may be ascribed largely to the difficult technique of culturing heart muscle.

The present paper presents the results obtained by us in the study of the process of secondary differentiation of explanted heart muscle.

EXPERIMENTAL METHODS

We used fragments of heart muscle of 10-day chick embryos, which were cultured in cells for 10-15 days. They were examined in the living condition, and after histologic treatment according to Bielschowsky and Bouquet.

Fig. 1. Differentiated heart muscle myoblast; 12-day culture of heart muscle of a 10-day chick embryo.

EXPERIMENTAL RESULTS

Myoblasts growing in tissue culture have served as experimental material for numerous researches on the most varied problems. The growth of myoblasts in tissue culture has been described in detail in many publications, for which reason we shall not enter into any detailed description of their undifferentiated extra-corporeal growth. As a general rule, the growth of embryonic heart tissue explants is characterized by proliferation of myoblasts, and only rarely by formation of symplasts, in which it is sometimes possible, with the greatest difficulty, to distinguish fibrils staining diffusely with silver by the Bielschowsky-Bouquet method. The myoblasts are little differentiated, whether after short or prolonged culture (10-15 days) outside the organism. This shows clearly that special conditions are required in order to achieve secondary differentiation of elements of embryonic cardiac muscle. We were successful, by prolonged culturing of heart muscle, in achieving secondary differentiation of cardiac myoblasts.

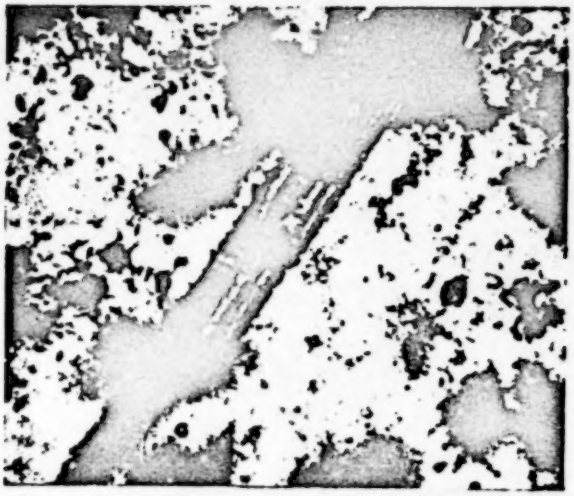

Fig. 2. Secondarily differentiated heart muscle fibers: 10-day culture of heart muscle of a 9-day chick embryo.

It will be seen from Figs. 1 and 2 that secondarily differentiated myoblasts are elongated bodies, sometimes fusiform; the nuclei are displaced towards one end of the cells. The myoblasts have a well-marked myofibrillar structure, with cross striations. These differentiated myoblasts are generally encountered in small numbers among the mass of proliferating undifferentiated cardiac myoblasts. This is evidence that some special conditions need to be satisfied in order to achieve differentiation of cardiac myoblasts: they appear only in very small regions of the myoblast culture, while the surrounding mass, including the myoblasts in direct contact with the differentiated ones, consists of undifferentiated cells of explanted myocardial tissue. All this shows that, as compared with achievement of secondary differentiation in tissue cultures of embryonic skeletal muscle, secondary differentiation of cardiac muscle explants remains a most complex and difficult process. Much painstaking and persevering work needs to be done in order to work out a method which would allow the ready initiation of the process of secondary differentiation of cardiac myoblasts.

SUMMARY

A 10-day chick embryo heart muscle was cultivated out of the organism. A secondary differentiation of myoblasts was obtained, which was characterized by development of myofibrillar structures with marked transverse striations.

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RESEMBLANCES AND DIFFERENCES BETWEEN HUMAN AND ANIMAL ORGAN-SPECIFIC ANTIGENS

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It has been shown by a number of authors [3, 5, 10, 11, 12, and others] that every organ possesses antigens specific to itself, in addition to general antigens characteristic of the given organism. Indisputable evidence of the existence of organ-specific antigens was afforded by the preparation of sera monospecific with regard to human organs [8].

The discovery of organ-specific antigens raised the question as to whether these antigens are identical for organs of analogous function of humans and of different animal species. It is known that the corresponding organs of different species of animals exhibit morphological and physiological resemblances.

The study of organ-specific antigens has yielded contradictory data. Some workers [1, 9, and others] reject the view that organ-specific antigens of different species resemble each other, and maintain that strict species-specificity prevails, while others [2, 14, 15] hold that organ specificity is not restricted by species differences, i. e., that the organ-specific antigens of different species are either identical or very closely related.

The majority of workers [3, 5, 10, 13, and others] admit the resemblance between organ-specific antigens, with the reservation that the antigens of some organs (brain, crystalline lens, posterior hypophysis) of different species are closely related, but of other organs much less so.

It thus seems that the question of the resemblance or the difference between the antigens of homologous organs of different species of animals and of humans has not yet received any definitive experimental answer. The present paper describes experiments directed towards this end.

Our objective was to determined whether there are any qualitative differences between human and animal organ-specific antigens.

For this purpose we prepared organ-specific immune sera against human organs, in rabbits. We examined these sera by the complement-fixation test with extracts of animal organs, and came to the same conclusion as many of our predecessors, that human and animal organs possess similar antigens. Our findings differed, however, insofar as we found that the reaction with extracts of animal organs was much weaker than of human organs. It remained to be shown whether this difference was a quantitative one only, or whether there exist qualitative differences between the antigens of different species.

EXPERIMENTAL METHODS

For the comparative study of organ-specific antigens we applied the reaction of binding of hemagglutinins, which we had previously used for the demonstration of human organ-specific antigens. This reaction is based on the secondary, nonspecific binding of α-agglutinins by specific antigen-antibody complexes.

This method permitted the examination of whole tissue brei, and not of extracts only. This was very convenient, as it gave us the opportunity of blocking heterogeneous antigens common to human and animal organs, which would interfere with the demonstration of specific antigens. Blocking of nonspecific antigens was effected by preliminary treatment of the tissues with various heterologous sera, in order to saturate the antigens.

We performed comparative experiments on human liver and spleen, and on the same organs of guinea pigs, dogs, pigs, and bulls. We used suspensions of the tissues in an equal volume of physiological saline for the reaction of binding of hemagglutinins. The organ-specific sera used for the hemagglutination reaction were diluted with 5 volumes of saline, and to 3 ml of diluted serum we added 0.3 ml of α-immune serum, diluted 1:10, and taken from a rabbit. The organ-specific serum so prepared was titrated against group A erythrocytes, undiluted, and in dilutions of 1:2, 1:3, 1:4, and 1:5. Serum diluted 1:5 usually gave a + or ++ reaction for agglutination of erythrocytes. The 50% tissue suspensions were measured by means of a pipet (0.5 ml portions) into agglutination tubes. These were then centrifuged for 10 minutes, and the supernatant solution was discarded. The centrifugate was taken for the experiments.

The experiments consisted of three stages.

In the first stage of the experiments we treated the tissue residues with various heterologous sera, for the preliminary blocking of heterogeneous antigens. Thus for the comparative study of human and animal spleen (Table 1) we used the following antisera: anti-spleen, anti-brain, anti-kidney, anti-stomach, and anti-myocardium.

The preliminary treatment of organ tissues consisted in addition to the centrifugates of 0.2 ml of the appropriate serum, which was mixed in with the brei, and the mixture was allowed to stand at room temperature for 30-45 minutes. It was then centrifuged, and the supernatant serum was titrated against Group A erythrocytes. A lowering of the titer of α-agglutinins in the serum was evidence that an immunological reaction had proceeded, whereas if there was no change in the α-agglutinin titer it was concluded that there had been no reaction.

The preliminary treatment was repeated with fresh portions of serum until all the heterogeneous antigens had been blocked, i. e., until the tissues gave no further lowering of the α-agglutinin titer of added serum.

We found that 4-5 additions of heterologous serum were required for full blocking of the common antigens of human organs, whereas 1 or 2 additions of serum sufficed for blocking antigens common to both human and animal organs.

The tissue breis were taken for further study after all these antigens had been fully saturated.

In the second stage, involving a specific reaction between organ-specific antigens and the corresponding sera, we added 0.1 ml portions of anti-liver serum to portions of human and animal liver tissue, mixed, and kept in the refrigerator for 15-16 hours.

The final, third stage consisted in assessing the extent of binding of α-agglutinins. The suspensions were centrifuged for 10 minutes at 2000-2500 r p m, and the supernatant serum was titrated against Group A erythrocytes.

EXPERIMENTAL RESULTS

Table 1 presents the results of comparative study of organ-specific antigens of human, guinea pig, dog, pig, and bull liver. It may be seen from this Table, which gives typical protocols of agglutination reactions, that preliminary treatment of human liver tissue with various heterologous sera does not remove its capacity of entering into specific reaction with anti-liver serum, as is shown by a regular fall in the α-agglutinin titer of the serum. Thus if the anti-liver serum initially agglutinated Group A erythrocytes at all dilutions, after it had been allowed to react with human liver tissue it scarcely agglutinated the erythrocytes at all. This is evidence that in the process of formation of the liver antigen-antibody complex practically all of the α-agglutinins were bound nonspecifically.

Quite different results were obtained with animal liver tissues previously treated with the same heterologous sera as were used for human liver tissue. The agglutinating power with respect to Group A erythrocytes was the same after anti-liver serum had reacted with animal liver as before. The unchanged α-agglutinin titer

TABLE 1

Results of Hemagglutination Reactions Performed in Connection with a Comparative Study of Human and Animal Liver Tissue

Expt. No.	Organ studied	Serum used for preliminary treatment	Serum used for specific absorption	Hemagglutination reaction with Group A erythrocytes of the serum after specific absorption, at dilutions				
				undiluted	1 : 2	1 : 3	1 : 4	1 : 5
	—	—	Anti-liver	+++	+++	++(+)	++	++
39	Human liver	Anti-spleen	»	+(+)	—	—	—	—
	Human liver	Anti-brain	»	+	±	—	—	—
	Guinea pig liver	Anti-spleen	»	+++	+++	++	++	+(+)
	Guinea pig liver	Anti-brain	»	+++	+++	+(+)	+	+
	Human liver	No treatment	»	—	—	—	—	—
	Guinea pig liver	No treatment	»	+(+)	+	±	—	—
	—	—	Anti-liver	+++	+++	+++	+++	++
44	Human liver	Anti-spleen	»	+	—	—	—	—
	Dog liver	Anti-spleen	»	+++	+++	+++	+++	++
	Dog kidney	Anti-spleen	»	+++	+++	++(+)	++(+)	++
	Dog spleen	Anti-spleen	»	+++	+++	+++	+++	++
	Human liver	No treatment	»	++(+)	+(+)	+	—	—
	Dog liver	No treatment	»	++(+)	+(+)	+	+	±
	—	—	Anti-liver	+++	+++	++	+(+)	+(+)
20	Human liver	Anti-spleen	»	++	+	±	—	—
	Human liver	Anti-spleen	»	++	+	±	—	—
	Human liver	Anti-brain	»	+(+)	±	—	—	—
	Human liver	Anti-stomach	»	++	±	—	—	—
	Pig liver	Anti-kidney	Anti-liver	+++	+++	++	+(+)	+(+)
	Pig liver	Anti-spleen	»	+++	++(+)	++(+)	++	+(+)
	Pig liver	Anti-brain	»	+++	+++	++(+)	++	+(+)
	Pig liver	Anti-stomach	»	+++	+++	++	++	+(+)
	Human liver	No treatment	»	+	—	—	—	—
	Pig liver	No treatment	»	+++	+	+	±	—
	—	—	Anti-liver	+++	++	+(+)	+	+
160	Human liver	Anti-kidney	»	+	—	—	—	—
	Human liver	Anti-myocardium	»	+	—	—	—	—
	Human liver	Anti-stomach	»	+	±	—	—	—
	Bull liver	Anti-kidney	»	+++	+(+)	+	+	+
	Bull liver	Anti-myocardium	»	+++	++	+(+)	+	+
	Bull liver	Anti-stomach	»	++(+)	++	+	+	+
	Human liver	No treatment	»	—	—	—	—	—
	Bull liver	No treatment	»	++(+)	+(+)	+	—	—

Note: +++, ++ (+), ++, +(+), +, ± represent decreasing degrees of intensity of the positive reaction; — represents absence of reaction.

Note: +++, ++(+), ++, +(+), +, and ± represent descending degrees of intensity of the positive reaction; — represents absence of reaction.

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TABLE 2

Results of Hemagglutination Reactions Performed in Connection with a Comparative Study of Human and Animal Spleen Tissue

Expt. No.	Organ studied	Serum used for preliminary treatment	Serum used for specific treatment	Hemagglutination reaction with Group A erythrocytes of the serum after specific absorption, at the dilutions:				
				undiluted	1:2	1:3	1:4	1:5
	—	—	Anti-spleen	+++	++(+)	++(+)	++	+(+)
15	Human spleen	Anti-liver	»	++	—	—	—	—
	Human spleen	Anti-brain	»	++	—	—	—	—
	Guinea pig spleen	Anti-liver	»	+++	++(+)	++(+)	++	+(+)
	Guinea pig spleen	Anti-brain	»	+++	+++	++(+)	++	+(+)
	—	—	Anti-spleen	+++	+++	+++	+++	++
45	Human spleen	Anti-liver	»	++	+	+	—	—
	Human spleen	Anti-brain	»	++	+	±	—	—
	Human spleen	Anti-stomach	»	+++	++	±	—	—
	Dog spleen	Anti-stomach	»	+++	++(+)	++(+)	++	+(+)
	Dog kidney	Anti-stomach	»	+++	++(+)	++(+)	++	+(+)
	Dog liver	Anti-stomach	»	+++	+++	+(+)	+(+)	+(+)
	Human spleen	No treatment	»	++	+(+)	+(+)	+	±
	Dog spleen	No treatment	»	+++	++(+)	++(+)	++(+)	+(+)
	—	—	Anti-spleen	+++	+++	++(+)	++	+(+)
30	Human spleen	Anti-brain	»	+	—	—	—	—
	Human spleen	Anti-stomach	»	+	—	—	—	—
	Pig spleen	Anti-brain	»	+++	+++	++(+)	++	+
	Pig spleen	Anti-stomach	»	+++	+++	++(+)	++	+(+)
	Bull spleen	Anti-brain	»	+++	++(+)	++(+)	++	++
	Bull spleen	Anti-stomach	»	+++	+++	++(+)	++	+(+)
	Human spleen	No treatment	»	—	—	—	—	—
	Pig spleen	No treatment	»	++(+)	+	—	—	—
	Bull spleen	No treatment	»	+++	++	+	—	—

Note: +++, ++(+), ++, +(+), +, and ± represent descending degrees of intensity of the positive reaction; — represents absence of reaction.

is evidence of the absence of immunologically specific reactions between animal liver tissues and anti-liver serum.

Human and animal liver tissues not subjected to preliminary treatment (see Table 1) enter into immunological reaction with anti-liver serum (fall in α-agglutinin titer); this reaction evidently proceeds as a result of the presence of similar antigens in human and animal organs.

The abolition of the capacity of animal liver tissue to enter into immunological reaction with anti-liver serum after blocking of nonspecific antigens with heterologous sera is in contrast to human liver tissue, which retains this capacity after repeated treatment with heterologous sera; this reveals qualitative differences between human and animal liver antigens.

Table 2 gives the protocols of comparative studies of human, guinea pig, dog, pig, and bull spleen tissue.

The spleen tissue was subjected to preliminary treatment with anti-liver, anti-kidney, anti-brain, and anti-stomach sera. We used anti-spleen serum with α-agglutinins for the specific reaction.

The experiments showed that spleen tissue of animals did not, after the preliminary treatment, react with anti-spleen serum (α-agglutinin titer unchanged). It follows that human spleen tissue resembles human liver in differing qualitatively from animal spleen tissues.

We have thus been able, by means of the method of blocking of heterogeneous antigens common to human and animal tissues, and using the hemagglutination reaction, to show that human organ-specific liver and spleen antigens differ qualitatively from those of the animals studied by us. This qualitative difference is apparently due to species-specific properties of the organ-specific antigens.

It is known that species-specificity of organisms is determined chiefly by the serum proteins. Proteins identical with serum proteins have been found in human liver and tumor tissues [6].

Our experiments show that species-specificity of organs is determined not only by the serum proteins, but also by organ-specific antigens.

The possibility is not excluded that the species-specificity of organs is due to a number of different antigenic complexes. It is possible that each antigen of a cell bears the characters of species-specificity.

SUMMARY

A comparative immunological investigation of the tissues of liver and spleen of man, guinea pig, dog, pig and bull was conducted. It was demonstrated with the aid of a method of blocking of heterogeneous antigens (common for organs of man and animals) and by the reaction of hemagglutinin binding that organ-specific antigens of man differ qualitatively from the corresponding antigens of animals. Organ-specific antigens, evidently, determine together with the other antigens the species to which the organ belongs.

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MORPHOLOGY AND PATHOMORPHOLOGY

FUNCTIONAL MORPHOLOGICAL CHARACTERISTICS OF THE STRUCTURE OF THE INTESTINAL MUCOSA OF RATS

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Physiological evidence of the periodicity of secretion of intestinal juice, first obtained by V. N. Boldyrev [1] in I. N. Pavlov's laboratory, has now been confirmed by the results of morphological studies.

M. I. Razumov [4] has shown that intestinal secretion is based on periodic accumulation of epithelial cells, in the form of elongated epithelial tubular processes at the tips of the villi, followed by shedding of these formations into the lumen of the intestine. The epithelium of the lateral walls of the villi is shed at the same time. As a result of these processes, the reticular stroma of the villi is divested of its epithelial covering for a certain time. The shed elements constitute the solid part of intestinal secretion, in which, according to G. K. Shlygin [8], S. Ya. Mikhlin [3], and L. S. Fomina [7], is contained up to 70-80% of the enzymes of intestinal juice. According to the observations of M. I. Razumov [4] the restoration of the epithelium of the villi proceeds through the stage of a symplast in dog's intestine; this was first described in the intestine of a growing carp (W. M. Smallwood and M. L. Smallwood [10]).

The object of our present research was to ascertain whether shedding of epithelial cells into the lumen of the intestine takes place in rats, and to find out whether the process has any distinguishing characteristics in this animal.

EXPERIMENTAL METHODS

Our experiments were performed on 40 rats, divided into several groups. The animals were killed at various times after feeding: 1) immediately after feeding (rats Nos. 33-35); 2) 2 hours after feeding (rats Nos. 30-32); 3) 7 hours after feeding (rats Nos. 27-29); 4) 24 hours after feeding (rats Nos. 1-23 inclusive); 5) 48 hours after feeding (rats Nos. 24-26); 6) under ordinary laboratory feeding conditions (rats Nos. 36-40) – excess food was given overnight, and the rats were killed at 9 a.m. the next day.

The animals were killed by decapitation, and were immediately opened up for sampling. Samples of duodenum, jejunum, ileum, appendix, and colon were taken, as well as of other viscera, for the purpose of assessing the general state of health of the animal. The material was fixed in Zenker's solution, ethanol, 10% formalin, embedded in paraffin and celloidin, and sections up to 6 μ thick were prepared. The sections were stained with hematoxylin-eosin, thionine, picrofuchsin, and tested for the Feulgen and Brachet reactions. Frozen sections were treated according to Foot for the staining of argyrophilic fibers.

EXPERIMENTAL RESULTS

Our studies showed that the structure of the intestinal mucosa of rats is distinguished by certain characteristic features. In contrast to the intestine of humans (N. Gundobin [2]), dogs (M. I. Razumov [4]), hedgehogs (J. Grimm [9]), and other animals, the duodenal mucosa of rats never forms circular folds. Circular folds are to be seen in the appendix of rats. The folds of the colonic mucosa cannot be considered to be Kerckring's folds,

as they are smoothed out when the colon is filled with fecal material.

Villi are to be found over the entire length of the duodenum and small intestine of rats. The villi are longest in the duodenum and the upper part of the small intestine. Within the ileum they are low, and have a cylindrical shape, or occasionally they appear as broad plates (in transverse sections). Villi are absent from the appendix and the colon.

The intestinal mucous membrane rests on areolar tissue, containing large numbers of argyrophilic fibers. These fibers are more densely distributed at the surface of the areolar tissue, immediately below the mucous membrane of both the glands and the villi. Side by side with the argyrophilic fibers in the central parts of the villi thin bundles of muscle fibers can be seen, constituting together with the argyrophilic fibers, the basis of the stroma of the villi. Slender collagen fibers may be distinguished in small numbers in the areolar tissue of the glands, and along the course of the blood vessels of the villi, where they belong to the adventitial elements of the vessel walls. Argyrophilic fibers may also be found in the areolar stroma of the mucous membrane of the appendix and the large intestine, where they are thicker than in the small intestine. Apart from this, slender collagen fibers appear in the mucous membrane of the appendix and large intestine, in small numbers.

Wandering cells, consisting in rats of lymphoid elements, mast cells, and eosinophils with round, annular nuclei, are to be found within the meshes of the reticular syncytium of the stroma of the villi and glands of all sections of the intestine. It is known that in some animals, such as the dog (M. I. Razumov [4]), the lymphatic system of the stroma of the villi consists of a central lacteal, the walls of which are lined with reticular cells, and in other animals by a formed lymphatic duct lined with endothelium, as we have observed in rabbits. The lymphatic system of the villi of rats consists of a system of channels in the reticulum of the stromal tissue. These channels become regular, endothelium-lined lymph ducts in the vicinity of the intestinal glands.

The surface of the areolar stroma of the intestinal mucous membrane is covered with a layer of secretory epithelium, which in the rat includes goblet cells. The intercellular spaces of this epithelial layer may, as in other species, contain wandering cells, such as lymphoid elements and eosinophils. The number of such cells is, however, considerably smaller in rats than in rabbits. Not more than 50 cells can be found per histological section of the epithelium of a whole villus of a rat. In rabbits, 300 cells or more can be seen in one field of vision of a section of the epithelium of the small intestine, at a magnification of 10 x 40. The wandering cells infiltrate from the areolar stroma of the mucous membrane, and are mostly to be found in the basal parts of the epithelial layer.

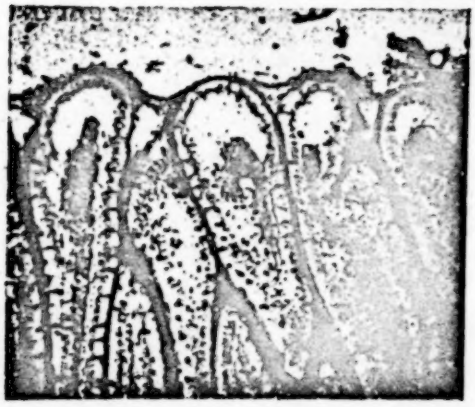

Fig. 1. Epithelial tubes forming at the tips of villi in the ileum of Rat No. 14.
Stained hematoxylin-eosin, magnification 12x10.

Our observations showed that functional changes in the mucous membrane, associated with digestive processes taking place in the intestine, have the most pronounced effects on the structure of the epithelial layer. It was also found that the structure of the epithelial layer varies for rats of different groups, killed at different times after feeding. As in dogs, various stages, connected with the process of accumulation and shedding of cells, can be distinguished in the state of the epithelium of rats. In the early stages of accumulation of cells the superficial layer of epithelium at the surface of the mucous membrane of the villi forms a continuous sheet, closely approximated to the reticular stroma. As cells continue to accumulate at the lateral surfaces of the upper parts of the villi, the epithelial layer sometimes becomes thrown into shallow folds. More frequently, however, detachment of epithelial cells from the lamina propria of the mucous membrane takes place without prior formation of folds. This process first affects only the tips of the villi, and then spreads to their lateral surfaces.

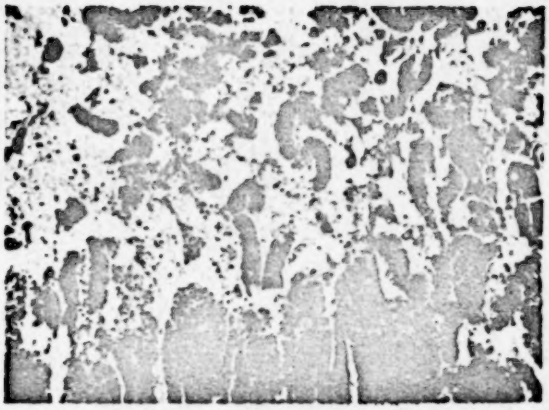

Fig. 2. Continuity of the epithelial layer interrupted, the upper parts of the villi denuded of epithelium; the lumen of the intestine contains shed tubules, fragments of tubules, and separate epithelial cells.
Stained hematoxylin-eosin, magnification 10 × 10. Rat No.34.

After the epithelial cells have been shed, protoplasmic strands emerging from the basal ends of the cells can be clearly seen in the gaps formed between the epithelium and the stroma when the cells were detached. Apparently these strands served for the attachment of the epithelial cells to the reticular stroma of the villi. With increase in the number of cells in the epithelium, and with its detachment at the tips of the villi, formation of epithelial tubules takes place (Fig. 1). These tubules are much smaller in rats than in dogs, as described by M. I. Razumov [4]. In both rats and dogs, the lumina of the tubules, and the gap remaining after their detachment, contain aggregations of granular protein, giving a positive reaction for ribonucleic acid. This granular formation was described by M. I. Razumov as being the fluid part of the intestinal juice. Isolated wandering cells, which had migrated from the reticular stroma of the mucous membrane were seen in the subepithelial granular substance of rats.

Shedding of epithelial tubules, their fragments, and of isolated cells into the lumen of the intestine supervenes at a certain stage of accumulation of cells in the epithelial layer (Fig. 2). As in dogs, the process spreads to the epithelium of the lateral surfaces of the villi. In such cases, the reticular stroma of the villi of rats, right down to the openings of the crypts, is denuded of epithelium, and is bathed in the contents of the intestine (Fig. 3).

Our studies showed that the nuclei of cells shed into the intestinal lumen of rats do not differ in their appearance and in their staining capacity, as well as in the distribution of chromatin within them, from those of the corresponding cells of the mucous membrane. The Feulgen reaction for deoxyribonucleic acid given by the nuclei of shed cells gives the same picture as is commonly seen in the cells of the mucous membrane of the intestine. We found that the shed cells are rich in alkaline and acid phosphatase, the distribution of which in the intestine of rats has been studied in detail by B. K. Skirko [5, 6].

Breakdown of the shed cells then takes place in the intestine, evidently as a result of the action of proteolytic enzymes of pancreatic juice, and structural changes are to be seen in the nuclei, which finally disappear. Aggregates of granular masses, staining intensively with eosin, are visible in the lumen of the intestine.

We found that shedding of cells into the intestinal lumen does not proceed simultaneously at all levels of the intestine, but takes place in a wave-like manner. Our observations showed that the structure of the epithelial layer of the intestine differs at different levels, for one and the same animal.

Our morphological observations show that the epithelial cells shed into the lumen of the intestine constitute the solid fraction of intestinal juice. In rats, the solid fraction consists of epithelial plasts, tubules, their

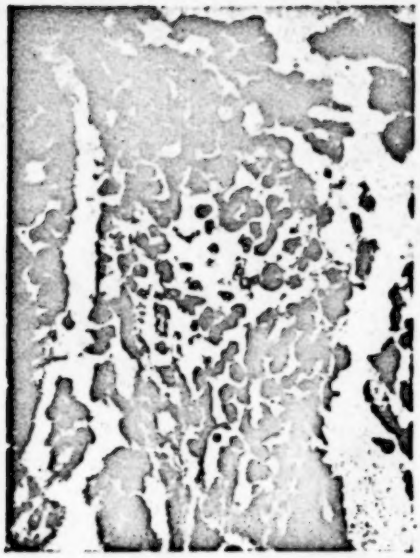

Fig. 3. Reticular stroma of the whole villus denuded of epithelium, and bathed in intestinal fluid, in which shed cells are visible. Stained hematoxylin-eosin, magnification 12 x 40. Rat No. 12.

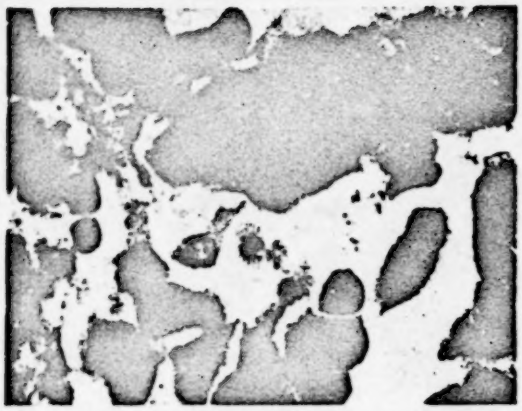

Fig. 4. Cells shed into the lumen of the intestine: epithelial and lymphoid cells, eosinophils. Stained hematoxylin-eosin, magnification 15 x 90. Rat No. 3.

fragments, and individual epithelial cells. Sporadic goblet and lymphoid cells may also be seen in the solid fraction. In contrast to dogs, the cells shed into the intestine of rats include individual eosinophils with round nuclei (Fig. 4).

We were unable to observe shedding of epithelial cells into the lumen of the appendix or large intestine, although in a few of the rats the intestine contained strands of cells with pyknotic nuclei and intensively staining cytoplasm. Such cells were in some cases present in a mass of foamy, apparently mucoid, intestinal content.

Our object in killing the animals at different times after feeding was to ascertain how the passage of food down the intestine affected the shedding process. Our morphological data, however, showed that the state of the epithelial lining varied in different rats killed at the same time after feeding.

SUMMARY

Intestinal mucous membrane was examined in rats, killed at various periods after feeding. It was established that there exists a regular disturbance in the continuity of the epithelial surface in the duodenum and small intestines of rats, i. e. there is an accumulation of epithelial cells on the villi in the form of epithelial tubes. Later these tubes and cells from the peaks and the sides of the villi are separated from the epithelial surface. According to the morphological data these separated cells form the solid fraction of the intestinal juice in the intestinal lumen.

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ORIGINATION OF NEUROBLASTS DURING POSTEMBRYONIC LIFE

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The question as to whether the intramural ganglions of the gastrointestinal tract possess a finite reserve of neuroblasts, laid down during embryogenesis, from which neurons later develop, or whether this reserve may be supplemented during extrauterine life, remains as yet unanswered.

A. A. Zavarzin and A. V. Rumyantsev [7] and N. G. Khlopin [17] reject the possibility of origination of neuroblasts during postembryonic life. A. I. Bubnova [2], M. I. Vekhova-Shandurova [4], N. M. Zhuk [6], N. G. Kolosov [11], S. I. Kolosova [12], Z. Ya. Tkachenko [16], Z. I. Khoros [18], and others do not mention this problem at all, and merely record the fact that neuroblasts are to be found in the ganglions of the Auerbach plexus of young humans and animals.

Isolated opinions on the division of neuroblasts during postembryonic life (T. A. Koblov [10]) were not supported by any convincing factual evidence.

T. I. Dekanosidze [5] believes that neuroblasts found "in the higher and lower levels of the nervous system" during the whole of the life-time of an individual "cannot represent the remnants of neuroblasts originating during the embryonic stage of life". The author admits the possibility that they may originate from "living matter."

EXPERIMENTAL METHODS

We studied the histological structure of the Auerbach plexus in the small and the large intestines of healthy cats and kittens of different ages, viz., newborn, 4 weeks to $1^1/_2$ months, 2-3 months, and adults (27 animals in all). Our observations were made on sections of whole preparations, treated by the methods of Bielschowsky-Gross, Avtsyn, and Spielmeyer. We studied the state of the nervous system of the intestine as a whole, and of tis nerve cells, fibers, receptors, and peripheral glia. The present communication deals with the origination of neuroblasts in the Auerbach plexus of young kittens.

EXPERIMENTAL RESULTS

Our studies of the histology of the Auerbach plexus of cats and kittens showed that the myenteric plexus contains, in addition to differentiated nerve cells of Dogel's Types 1 and 2, some nerve cells in the early stages of differentiation. Their morphological features are the same in all age groups. They are oval, round, or triangular cells, often concave on one side, dimensions from 6 x 8μ, 10 x 8μ, 12 x 6μ to 18 x 10μ, 20 x 22μ. The nuclei are oval, angular, or crescentic, with 1-2 nucleoli, dimensions 4-6-8μ, less frequently 10 x 8μ, 12 x 4 μ, always situated peripherally.

The cytoplasm of these cells is finely granular, staining weakly, and often containing slender neurofibrils. Nerve processes are either absent, or are in the early stages of formation, as short, pointed cytoplasmic processes

(incomplete impregnation of the processes was excluded). These were the least differentiated cells, and we shall hereafter refer to them as neuroblasts.

Other cells of this class were more highly differentiated, and represented transition stages between neuroblasts and mature neurons; their neurofibrillar network is more developed, and is as a general rule concentrated as a ravelled mass at the center of the cell, and they usually possess thin (diameter less than 1μ) processes, which disappear in the stroma of the plexus soon after emergence from the cell. The number of neuroblasts present in the Auerbach plexi is less than of differentiated nerve cells, in all age groups. The number of neuroblasts rises gradually from birth, during the first months of life; more are found in kittens aged from 4 weeks to 3 months than in newborn kittens. Individual ganglions of the plexus of 2-3 months-old kittens consisted exclusively of neuroblasts, which was never encountered in newborn kittens. There are fewer neuroblasts in adult cats than in kittens.

These findings are incompatible with the view that the ganglions of the Auerbach plexus possess a fixed reserve of neuroblasts, laid down during embryogenesis, which is depleted as they undergo differentiation to neurons.

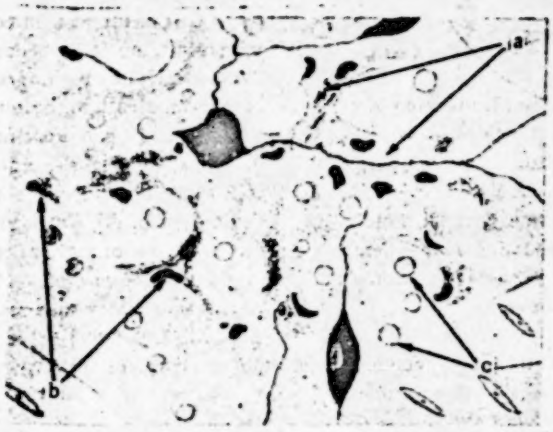

Fig. 1. Myenteric ganglion of the small intestine of a newborn kitten. Semidiagrammatic representation. Whole preparation. Impregnated according to Bielschowsky-Gross. Oil immersion.
a) Regions of differentiation of neuroglial syncytium; b) neuroblasts; c) peripheral glia nuclei.

From what do the neuroblasts originate? From our study of the peripheral glia of the Auerbach plexus we received the impression that they originate from the glial syncytium.

The peripheral glia of the ganglions of the Auerbach plexus of the intestine of cats and kittens is a syncytium, i. e., it consists of numerous nuclei embedded in a protoplasmic mass. The dimensions and the shape of the nuclei are identical in all age groups. With increasing age the number of nuclei rises, by amitotic division, and the protoplasmic mass of the neuroglial syncytium becomes firmer. The syncytial nuclei are, as a general rule, round or oval, and are located compactly. Individual parts of the syncytium differ from the above description in the nature of the nuclei and protoplasmic mass. In these regions, which may be called regions of differentiation of the neuroglial syncytium, the nuclei are more widely spaced, and closely resemble the nuclei of neuroblasts. Similarly to the nuclei of neuroblasts, they are of an irregular oval or angular shape, or are crescentic, and they measure 4-6-8μ. The finely granular, weakly-impregnating syncytial protoplasm forms a complicated pattern around the nuclei, with round vacuoles and narrow channels, i. e., it forms a sort of "nucleo-protoplasmic territory", from which arises the individual cellular element (Fig. 1). Slender neurofibrils can be seen in the cytoplasmic parts of the cells thus segregated (Figs. 2 and 3). Some of the above-described neuroblasts are usually located adjacent to these formations (see Fig. 1).

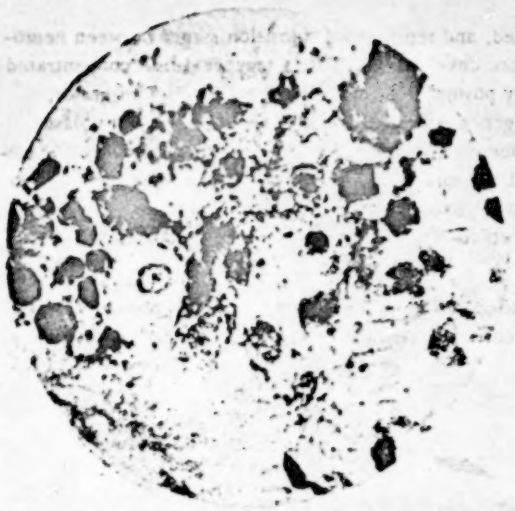

Fig. 2. Myenteric plexus of the small intestine of a newborn kitten. Photomicrograph. Neuroblasts segregated from the neuroglial syncytium. Syncytial continuity between the cells is still preserved, in the form of narrow isthmuses. The fine granular protoplasm of the segregating cells contains slender neurofibrils. Whole preparation. Impregnated according to Bielschowsky-Gross. Oil immersion.

A certain degree of correlation between the number of neuroblasts and the degree of differentiation of the neuroglial syncytium is observed at different ages. Regions of differentiation of neuroglial syncytium are particularly frequently encountered in newborn kittens, in which the number of neuroblasts is small. Individual ganglions of the Auerbach plexus of newborn kittens consist exclusively of differentiating syncytium containing solitary neuroblasts. In kittens aged from 4 weeks to 3 months regions of differentiation are less frequently encountered than in newborn kittens, and the number of neuroblasts in the ganglions rises; individual ganglions consist exclusively of neuroblasts. Regions of differentiation of the syncytium are less frequently seen in adult cats, and the number of neuroblasts in them is smaller than in kittens.

It is of interest, in connection with the above, to consider the embryogenesis of intramural ganglions. Evidence against the migration theory is afforded by certain observations, pointing to the origination of the intramural ganglions in situ, during the process of development of the organ (Remak [15], Veber [3], S. I. Matveeva [13]). Keining [8] showed that the neuroblasts of differentiating intramural ganglions of the esophagus of a 4-day chick embryo are indistinguishable from elements of the mesenchymal syncytium, similarly to which they retain syncytial interconnections. L. I. Belenko [1] remarked that the intramural ganglions of the vascular adventitia of 3-5 months human embryos represent "primitive anlage of syncytial structure".

It may, in the light of these literature references and of our own observations, be supposed that the neuroglial syncytium retains the capacity during postembryonic life of segregating and separating cellular elements, which then undergo differentiation to yield mature neurons.

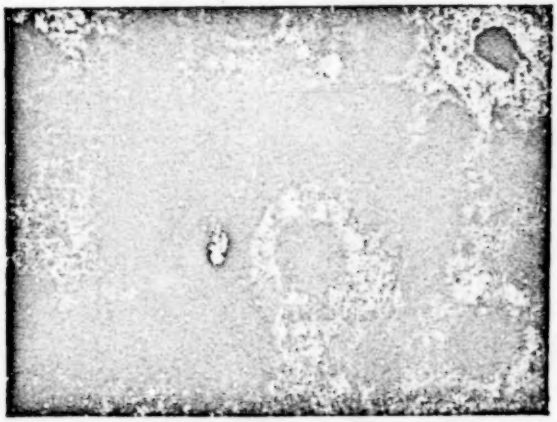

Fig. 3. The same preparation as in Fig. 2. Magnification x 1200.

The peripheral glia of the ganglions of the Auerbach plexus of newborn kittens appears to possess the greatest potential for differentiation of neuroglial syncytium. Intensive delimitation of neuroblasts is not, however, observed at this stage, which may be related to the presence within the ganglions at the time of birth of a sufficient number of differentiated nerve cells for the proper functioning of the nervous system of the intestine at this age.

In kittens aged from 3 weeks to 3 months, over which period growth and differentiation of the intestinal wall as a whole take place, in relation to the growth of the animal and to its transition to a different mode of nutrition, we observed an increase in the number of neurons in the ganglions of the plexus, due to differentiation of neuroblasts. In turn, the number of neuroblasts rises, owing to an intensive process of their delimitation from the neuroglial syncytium. The number of regions of differentiation of the syncytium must naturally become smaller, and the number of separated neuroblasts must increase, as a result of this process.

In adult animals, which have largely completed the process of growth and differentiation of the intestine, differentiation of the neuroglial syncytium is less frequently encountered, and the number of neuroblasts is smaller than in kittens.

These are our results. They reveal the source and the pathways of origination of neuroblasts in the intramural ganglions of the intestine in the postembryonic period of life of an animal. This finding is of great biological importance, since it elucidates the question of the regeneration of the intramural nervous system of the digestive tract, and provides indirect evidence of regeneration of neurons after their destruction in a number of pathological conditions. It would, in this connection, be of considerable interest to examine the peripheral glia of the Auerbach plexus in such pathological conditions of the gastrointestinal tract as are associated with mass destruction of neurons and with increase in the number of neuroblasts.

SUMMARY

Auerbach's plexus was studied histologically in the intestines of 27 healthy cats and kittens. Besides the differentiated neurons, neuroblasts were revealed in the ganglia of the intermuscular plexuses. In animals the number of these neuroblasts gradually increases during the first several months of life.

Peripheral glia in the plexus ganglia have 2 variations of the neuroglial syncytium. The first is in the form of a protoplasmatic mass with scattered nuclei, while the second is in the form of nuclear-protoplasmic areas with a tendency to separation of the cellular elements. The latter are morphologically similar to neuroblasts. The intensity of the process of separation of neuroblasts from the neuroglial syncytium is connected, in the author's opinion, with the functional activity of the digestive tract.

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STATE OF SYNAPTIC STRUCTURES AT NERVE CELLS OF THE SPINAL CORD OF IRRADIATED RATS

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The first convincing evidence of the effect of irradiation of frogs with x-rays was provided by L. R. Tarkhanov [19, 20]. It was later shown that radioactive substances cause degenerative changes in the nerve cells and fibers of the central nervous system [3]. Other authors [24, 26], however, reported that x-rays have no effect on nervous tissues, which remain intact after irradiation of the organism.

L. R. Tarkhanov's views were supported and further developed by M. I. Nemenov [16], F. P. Malorov, M. I. Nemenov and L. F. Vasileva [13], E. I. Bakin [1], N. N. Lifshits [12], R. Liman, P. Kupalov and W. Scholz [25], D. G. Shefer [21], P. F. Minaev [14, 15], and others, who found that the nervous system is quite sensitive to penetrating radiation. M. I. Nemenov [16] definitely established that x-rays adversely affect cortical activity. D. G. Shefer [21] showed that irradiation caused degenerative changes in the cells of the cerebral cortex and of the subcortical ganglions.

These divergent views on the sensitivity of the nervous system to the action of radiant energy are also reflected in the textbooks. V. V. Efimov [6] classes nervous tissue as being the least sensitive to radiation of all the tissues and cells of the body. While B. N. Tarusov [18] concedes on the one hand that the central nervous system is very sensitive to direct irradiation, on the other hand he considers that "the tissues of the nervous system are best of all protected against secondary toxic products formed during irradiation."

Of recent times, morphological data have been added to clinical and physiological observations indicative of the sensitivity of the nervous system to x-rays.

V. V. Portugalov et al. [17] have shown that irradiation of animals causes damage to the nerve cells of different levels of the brain, and of the sympathetic and spinal ganglions, and leads to changes in nerve fibers and receptors.

A. G. Beglaryan, K. A. Kyandaryan and S. A. Papoyan [2] came to the conclusion that irradiation affects receptor nerve fibers and cells of spinal ganglions (crenellation, hyperchromatosis, central chromatolysis). They also found changes in the lateral horns of the spinal cord (karyocytolysis, ghost cells), and, to a lesser degree, in the cells of the higher levels of the central nervous system.

The least affected were the cells of the cerebral cortex, which is, in the opinion of these authors, due to differences in the functional state of different parts of the central nervous system.

V. V. Kupriyanov [10, 11] subjected dogs and cats to irradiation, and found significant changes in the cardiac receptors and in the intramural nerve cells of the heart; he observed degenerative changes in the pericellular formations.

Irradiation restricted to the temporal or frontal lobes of the brain of apes was followed by acute inflammatory or necrotic processes in the irradiated parts of the brain, according to the amount of radiation administered [22]. Changes in the glia cells of irradiated apes were also observed [23].

A. D. Zurabashvili and B. R. Naneishvili [8] examined the condition of certain parts of the brain and the spinal cord of 4 irradiated dogs and 4 irradiated apes. They came to the conclusion that the central nervous system, consisting as it does of highly differentiated cells, is sensitive to irradiation.

The impression given by this short survey of the literature is that nervous elements are very sensitive to irradiation with x-rays. One may agree with N. A. Kraevsky's view [9] that the alleged insensitivity of the nervous system to radiant energy was based on superficial morphological observations.

The question of the effect of x-rays on the nervous system is, of course, still far from being finally elucidated; in particular, virtually no attention has been paid to the state of the synaptic structures, which are abundantly distributed around the neurons of the central nervous system.

For the explanation of the processes of stimulation and inhibition taking place in the central nervous system, present-day physiology attaches great importance to the function of the complex synaptic apparatus distributed around nerve cells and their protoplasmic processes (dendrites). B. A. Dolgo-Saburov [4, 5] has, on morphological grounds, advanced the suggestion that the concept of the neuron as a structural and physiological element of the reflex arc should be extended to include its surrounding neuroglia and blood capillaries. It was found [4, 5] that these elements are also supplied with synaptic structures, which appear to be terminations of nerve cell dendrites and axons (i.e., of conducting systems).

EXPERIMENTAL METHODS

We examined the condition of synaptic structures around spinal cord cells of rats which had been subjected to x-ray irradiation, at a dosage of 450-600 r, causing acute radiation sickness. The spinal cord of 5 rats killed on the 5-12th day after irradiation was studied in complete serial sections. The sections were impregnated by D. I. Deinek's modification of the Golgi method. This procedure gives a very clear picture of normal and pathologically changes synaptic structures at nerve cells and their dendrites. The capillary network is also well shown; for greater contrast we stained the capillaries with iodoeosin.

EXPERIMENTAL RESULTS

We compared our observations with those of A. D. Zurabashvili [7] and of B. A. Dolgo-Saburov [4], which describe the structure and distribution of synaptic structures of nerve cells of the spinal cord of normal rats. The terminal interneuronic apparatuses connecting spinal cord nerve cells of rats – the synapses – are normally in the form of slender ringlets or loops situated on the body of the cell and on the dendrites [4, 7]. Their number is fairly large, and their dimensions vary, but in general they give the impression of being extraordinarily thin. The synaptic ringlets are sometimes supplied by short preterminal segments of afferent nerve fibers, being axons of another nerve cell. Some of the synaptic structures are located between cells. These synapses, which had earlier been termed "free synapses" by Lorente de No, are in fact, according to B. A. Dolgo-Saburov [4, 5], terminations of nerve fibers at nerve cells and their dendrites which had not fallen within the plane of the section, at neuroglia cells, and, finally, at the walls of the multiplicity of capillaries which ramify in this region.

Many cells are literally covered with hypertrophied synaptic structures (Figs. 1 and 2). Some of these terminations lie close to the cell, within the perineural space. Synaptic terminations, including those located at interdendritic bridges between cells, undergo enlargement. Many synaptic structures not apparently connected with cells or their processes ("free" synaptic structures, according to the old terminology) are visible in the field of vision; many of these are also hypertrophied, and are located on the walls of capillaries (see Fig. 3). It may be concluded that all types of synaptic structures are affected: axosomatic, axodendritic, and axovasal, i. e., located at the walls of capillaries.

The morphological picture of the reactively modified synaptic terminations is distinguished by its lack of uniformity. The structures vary from barely perceptible enlargements and swellings of the synaptic loops to filled-in loops, many times greater than the original ones, and with indistinct contours. There are also a large number of intact synaptic structures.

Apart from the forms described, there are also more considerably modified synaptic structures; these have undergone transformations into swollen, mace-like forms (Fig. 4), or into plates of irregular shapes. These

synaptic terminations do not have a uniform structure. They contain vacuoles and granulations, their contours are irregular, and the perisynaptic fibers are split in places (see Figs. 1 and 2).

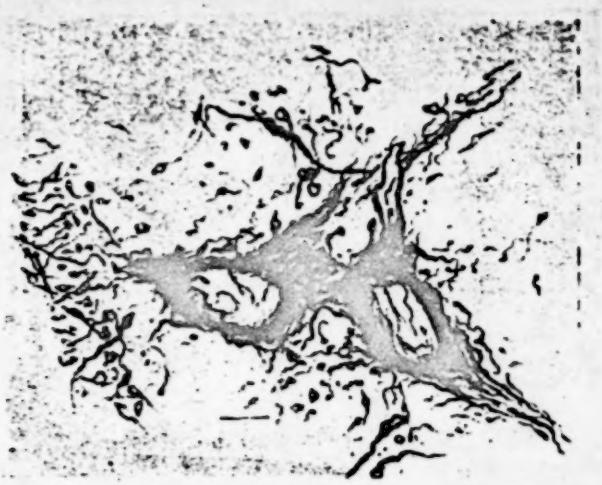

Fig. 1. Cervical segment of the spinal cord. Hypertrophied synaptic structures on the bodies of nerve cells and on their dendrites.
The synaptic structures seen to the left of the drawing are located on the walls of a capillary. Impregnated according to Golgi-Deinek. Oil immersion.

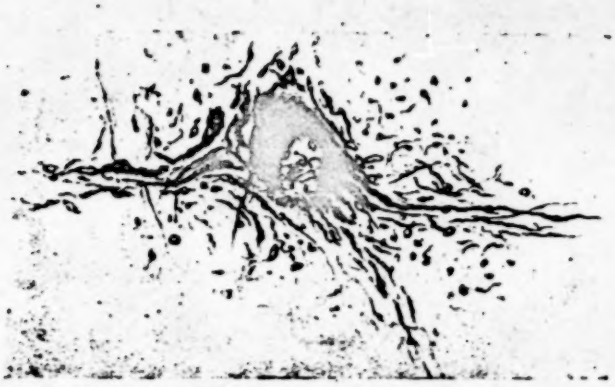

Fig. 2. Lumbar segment of the spinal cord. Hypertrophied synaptic structures on the body and the processes of a nerve cell. Impregnated according to Golgi-Deinek. Oil immersion.

It cannot be maintained that the above changes in synaptic structures are specific effects of radiation damage to the nervous system. It is known that the nervous system reacts fairly uniformly to different pathologically inadequate stimuli. Similar, although less pronounced, changes in synaptic structures at nerve cells of the spinal cord of cats, following ligation of the portal vein, have been described by B. A. Dolgo-Saburov [5].

The reactively modified synaptic structures represent an adaptive reaction. The neuron, of course, reacts as a unit to any agents acting on it, but the visible structural changes are evidently most readily observed

at its terminations (receptors, pericellular apparatuses, synaptic formations at cells of the central nervous system).

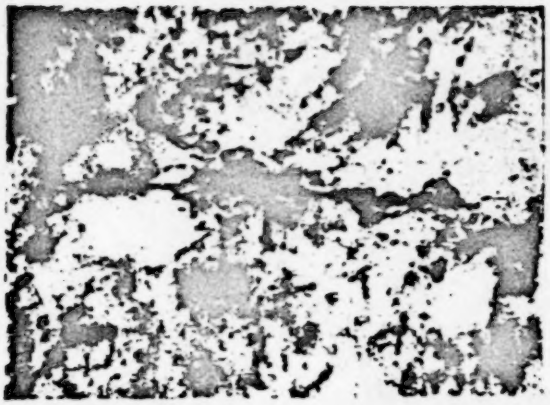

Fig. 3. Cervical segment of the spinal cord. Hypertrophied axovasal terminations on the wall of a blood capillary. Photomicrograph. Impregnated according to Golgi-Deinek. Oil immersion.

Fig. 4. Cervical segment of the spinal cord. Two very enlarged synaptic terminations, in the form of plates (one of them with a pre-synaptic fiber), may be seen at the upper end of the cell, at its axon root. Photomicrograph. Impregnated according to Golgi-Deinek. Oil immersion.

Apparently different parts of a neuron vary in their morphophysiological properties. This conclusion may be drawn from published data [8, 10, 11] and from the results of our experiments. The presence of intact synaptic structures side by side with reactively changed ones is striking evidence of the plasticity of the nervous system, which is responsible for maintaining the level of vital processes at the optimum for the given conditions.

The significance of our results is that they provide further support for the views of those authors who hold that the nervous system is highly sensitive to penetrating radiation. Apart from changes in the nerve cell bodies and their processes (which were observed by us), the greatest interest attaches to our findings on the reactivity of synaptic structures, to which there are very few references in the literature. It is known, for example, that irradiation of different parts of the central nervous system causes inhibition or stimulation of their activity, according to the dosage given [14].

These processes cannot proceed without the participation of the synapses, which connect one neuron with another. The changed synaptic structures may possibly interfere with proper functioning of reflex arcs, thus causing localized and generalized disturbances.

SUMMARY

Various changes were observed in synaptic structures following irradiation in the dose of 450 to 600 r. These structures were changed from delicate rings into button-like, mace-like structures and nodules of large size. All types of synaptic structures are subjected to hypertrophy – axosomatic, axodendritic and axovasal. However, there is a considerable number of synaptic structures which remain intact. This points to the high plasticity of the nervous system. Changed synaptic structures, probably, cause alteration of reflexes with ensuing local and general disturbances.

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METHODS

SIMULTANEOUS MEASUREMENT OF ELECTROMOTIVE FORCE AND CURRENT STRENGTH (QUANTITY OF ELECTRICITY) AS A METHOD OF INVESTIGATION OF BIOELECTRIC PHENOMENA

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Following the work of V. I. Chagovets (1896, 1903), the concept of biocurrents originating in a peculiar concentration element formed between injured or excited and intact or "resting" areas of tissue has persisted in modern electrophysiology.

Such a generator, like any generator in general, can be characterized either by current strength or by electromotive force (e.m.f.) – voltage in the external circuit of the generator – and their changes under various conditions. In order that certain current strength and voltage be maintained in the external circuit of the generator the generator must transform some form of energy into electric energy; in other words, energy transformation and its expenditure occur only when the generator is working: current flows in the external circuit and the difference of potential at the generator terminals tends to zero. It is for the maintenance of this difference that the expenditure of transformed energy is, in the final analysis, required. The quantity of generated electricity Q characterizes the work of the generator. This work can be calculated according to the formula: $A = ItE$, where A – work; I – current strength; t – time; E – e m f; $It = Q$ where Q – quantity of electricity.

In chemical sources of current it is the heat of the chemical reaction or osmotic work involved in equalizing ionic concentrations that is transformed. The question naturally arises as to the nature of energy which is transformed into biocurrent on excitation and injury of tissue. It may be supposed that such energy in any tissue of a living organism must be chemical, i. e., energy of metabolism, since it has been shown in a number of investigations that a connection exists between the state of metabolism in the tissue and bioelectric phenomena.

The main method used in modern electrophysiology is the measurement of e m f of tissue as a more precise index than current strength and independent of changes in the internal resistance of the generator (tissue) when the compensatory method of e m f measurement is employed.

Using ordinary nonpolarizing electrodes ($Zn-ZnSO_4$) with agar cocks with considerable resistance (many thousand ohms) and completing the circuit for very brief periods with balanced measurement of e m f conditions are created under which no appreciable current flows in the generator circuit and, therefore, the generator performs no work. In order to maintain these conditions modification of the methods used in studying biocurrents is required.

In an attempt to study the relation of injury currents (e m f) to tissue metabolism (energy being transformed), and taking the hypotheses cited above as a basis, the following points were investigated in the present work: 1) relation of fall in e m f and in strength of injury current to electrode resistance; 2) relation between some aspects of tissue metabolism and the quantity of electric energy generated by the tissue.

METHODS

The experiments were performed on the sartorius muscle of autumn and winter frogs. The pelvic end of the muscle was injured by a special clamp. The muscle was placed in a paraffin bath so that the injured end was in one chamber of the bath and the intact end in the other. Each chamber was filled with Ringer solution or solution of the substance whose effect was being studied. The chambers were divided by a partition consisting of a mixture of paraffin with paraffin oil. Injury current was led off by nonpolarizing electrodes ($Zn-ZnSO_4$) with resistance of 800, 1,500, 2,200, 11,000, 50,000 ohms. Low resistance electrodes (800 ohm) were prepared according to the scheme described by A. V. Lebedinsky and A. S. Mozzhukhin [3]. In order to obtain electrodes with resistance of 1,500, 2,200, 11,000 and 50,000 ohms the original 800 ohm electrodes were connected with appropriate resistances. To measure the current strength (quantity of electricity generated), the muscle was included by means of appropriate electrodes in the circuit of a mirror galvanometer with resistance of 2,000 ohms, graduated with respect to current strength, for 6 hours. To measure the e.m.f. the muscle was included in the circuit of a balanced installation in which the same mirror galvanometer served as the "zero" instrument.

The energy generated by the muscle was calculated [3] according to the formula:

$$E=0.24\left(QU_{cp}+\frac{QU_{cp}}{5}\cdot 2\right)$$

where $Q = I_{cp}t$,

where E – energy in small calories; Q – quantity of electricity; U – e m f; I – current strength; t – time; Ucp and I cp were calculated as a mean of a number (7) of measurements during the 6 hours of experiment. The value of liberated energy was expressed in microcalories for the sake of more convenient comparison with the majority of literature data.

The influence of impairment of carbohydrate-phosphorus metabolism was studied in experiments in which the intact end of the muscle was poisoned by substances listed in the table. The concentrations indicated in the table are, according to data available in the literature (Belitser [1], Parnas, 1940 and others) the most effective in disturbing carbohydrate-phosphorus metabolism in skeletal muscle.

In most of the experiments the intact portion of the muscle was subjected to the action of the various substances throughout the whole experiment (6 hours). In some experiments the intact portion of the muscle was exposed to the action of monoiodoacetate solution for only 30 minutes; the substance was then replaced by Ringer solution or solution of vitamin B_1, which is designated in Fig. 2 as "washing".

RESULTS

Figure 1 shows changes in strength of current generated by the muscle with various resistance of the external circuit. The greatest current strength is observed when this resistance equals 3500-4200 ohms (electrode resistance 1500-2200 ohms). Measurement of resistance in the area of the muscle lying between the electrodes according to A. V. Lebedinsky's scheme [2] yields a value equal to 3000-4000 ohms. These data show good correspondence with the well-known fact that the generator performs most work (and hence the greatest transformation of energy) when the internal and external resistances of a circuit are equal. This fact was utilized in experiments in which the quantity of electric energy generated by the muscle under conditions of impaired carbohydrate-phosphorus metabolism was being studied. The results of these experiments are presented in the table and in Fig. 2. The data show that impairment of carbohydrate-phosphorus metabolism by one or other enzyme poison lowers the electric energy generated by the injuered muscle.

It follows from the table and Figs. 1 and 2 that, on the one hand, under certain conditions the maximal electric energy liberated by the injured muscle can be measured, and, on the other hand, the value of energy thus liberated can undergo a change when carbohydrate-phosphorus metabolism is disturbed. Changes in generation of electric energy are most marked when the muscle is poisoned by monoiodoacetate, fluoride and hydrazine (which interfere with the reactions connected with transfer of phosphate to the ATP-phosphocreatine system), 2,4-dinitrophenol (which interferes with conjugated oxidative phosphorylation) and veratrine (which accelerates breakdown of phosphorus compounds).

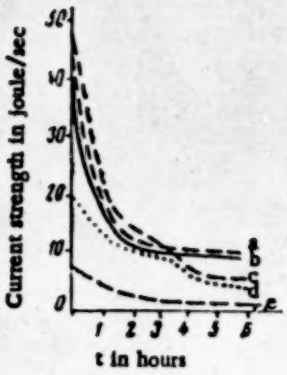

Fig. 1. Strength of current generated by the muscle with various resistance in the external circuit.
A) 4200 ohm; B) 3500 ohm; C) 2800 ohm; D) 1300 ohm; E) 52000 ohm (electrode resistance; A) 2200 ohm; B) 1500 ohm; C) 800 ohm; D) 1100 ohm; E) 50000 ohm).

It can thus be assumed that during generation of injury biocurrent energy liberated in the process of carbohydrate-phosphorus metabolism is used up.

There is a concept at present which proposes that liberation of energy in muscle tissue takes place by way of the ATP-phosphocreatine system. It is possible that this system is the direct energy source in the generation of biocurrent. It may be supposed that both glycogen breakdown and respiration area energy sources in the resynthesis of this system. The energy potential of the ATP-phosphocreatine system exceeds several times the quantity of energy required for the generation of injury current. However, formalin-fixed muscle which is, apparently, free of even traces of metabolic processes, still gives some difference of potential. But this difference of potential is not great (one half to one third of the e m f of control muscle) and the formalin-treated muscle cannot produce any significant quantities of electric energy (the total amount of electric energy generated by control muscle is 8-10 times greater); the formalin-treated muscle behaves as a discharged accumulator (element): when it is switched in by way of low resistance electrodes it shows, in the first few moments, some difference of potential and current strength but these values decrease markedly in the course of a few seconds. All these considerations not only do not refute the dependence of injury current on metabolic processes but would even appear to support this hypothesis (A. S. Mozzhukhin [4]).

Quantity of Electric Energy Liberated by Injured Muscle During 6 Hours Under Various Conditions of Impairment of Carbohydrate-Phosphorus Metabolism (all figures are average of 10 experiments)

No. of experiment	Enzyme poison or metabolite	Energy liberated in 10^{-3} microcalories per 100 mg muscle	Concentration of poison in which the intact portion of muscle was immersed
1	Control	1.0	–
2	Sodium monoiodoacetate	0.4	0.001 M
3	Sodium fluoride	0.5	0.02 M
4	Hydrazine sulfate	0.35	0.02 M
5	2,4-dinitrophenol	0.35	1:10 000
6	Sodium arsenate	1.0	0.02 M
7	Veratrine	0.4	1:10 000
8	Sodium cyanide	0.8	0.02 M
9	Phlorizin	0.8	0.01 M
10	Glyceraldehyde	0.65	0.02 M
11	Sodium pyruvate	1.1	0.02 M
12	Sodium lactate	0.95	0.02 M
13	Sodium sulfite	0.8	0.02 M
14	Formalin	0.1	20%

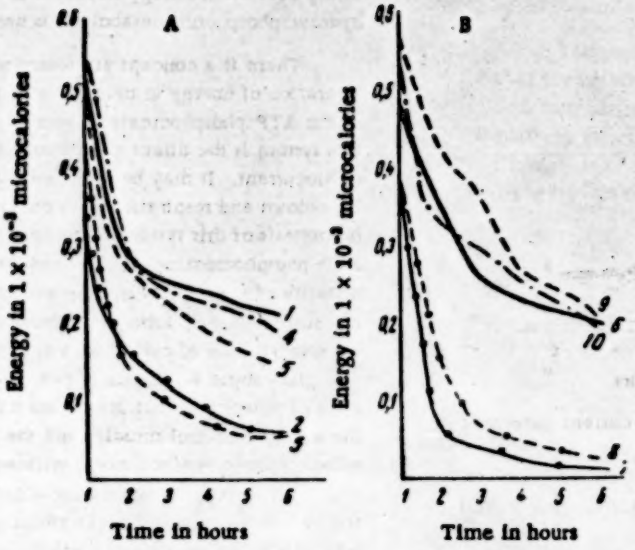

Fig. 2. The effect of poisoning isolated muscle by agents which impair carbohydrate-phosphorus metabolism on the quantity of energy generated (separate experiments with initial current strength of 7.5μ A and e m f of 50 mv).
Curves: A) 1) control, 2) monoiodoacetate poisoning with Ringer solution "washing", 3) phlorizin poisoning, 4) monoiodoacetate poisoning with vitamin B_1 solution "washing", 5) 2,4-dinitrophenol poisoning. B) 6) cyanide poisoning, 7) veratrine poisoning, 8) hydrazine sulfate poisoning, 9) lactate treatment, 10 arsenate poisoning.

It is possible that those small e m f and current values which are observed in formalin treated muscle are determined by liberation of structural energy or represent an accumulation of metabolic energy, or formalin may "fix" the differences of ionic concentrations in the muscle and the preparation represents a concentration element without continuously maintained difference of concentrations which are quickly equalized. Whatever the situation, metabolism evidently accounts for 0.9 of all the energy used in the generation of electric energy.

Simultaneous measurement of the quantity of electricity generated by muscle and of the e m f thus provides the opportunity for calculating the energy used in maintaining the potential difference between the injured and intact areas of tissue. Decrease in electric energy generated by the injured muscle when its carbohydrate-phosphorus metabolism is impaired proves that injury current and e m f are related to metabolism.

SUMMARY

Simultaneous measurement of the quantity of electricity generated by the muscle and of e m f gives the opportunity to estimate the energy which is used for maintenance of the difference of potentials between the injured and intact areas of tissue. Reduction of the value of electric energy, generated by the injured muscle, when the processes of carbohydrate-phosphorus m tabolism are disturbed proves the relationship of generation of biocurrent of the injured area to the condition of metabolism.

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* In Russian.

SPECIFIC PRECIPITATION REACTION IN AGAR BY THE OUCHTERLONY METHOD

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It has been known for some time that addition of antigens to agar containing the appropriate antiserum is accompanied by formation of lines of precipitate. Oudin [8, 9] carried out detailed studies on diffusion of antigens in the gel and conditions of formation of precipitates upon interaction of antigens and antibodies. He showed that the number of precipitation lines formed corresponded to the number of antigen-antibody systems participating in the reaction. In Oudin's method the specific precipitation reaction is carried out in test tubes with agar in which the antigen and antibody diffuse towards each other. Ouchterlony [6] modified this reaction suggesting that instead of one diffusion center for antigen two such centers be used, thus enabling direct comparison of two antigen preparations. The reaction was carried out in Petri dishes, 90-96 mm in diameter, with a thin layer of 1.5-2% agar (6-8 ml) poured on the bottom, dies were then placed in position, another layer of agar (16-18 ml) was poured over them after which the dies were removed carefully and the resulting hollows of 0.3-0.5 ml capacity were filled with antigens and antiserum. The dies were made of plastic in the shape of parallelepipeds measuring 12 × 12 × 12 × 3 mm; sometimes stoppers from penicillin vials were used for this purpose. The placement of the holows can vary depending on the number of participating ingredients. Experiments can be carried out in dishes with three and more hollows. Figure 1 shows the approximate placement of hollows in an experiment with 5 ingredients.

The agar used in the reaction must be transparent and it was therefore filtered, while hot, through cotton wool after precipitation by 0.5% calcium chloride. The solidified agar was cut into small pieces (about 1 mm^3) and washed in running water for 72 hours. Prior to pouring the molten agar into the dishes, NaCl was added to it (final concentration 0.8%), as well as merthiolate 1:10,000 and methyl orange 2:100,000. Merthiolate prevents bacterial growth, while methyl orange increases the contrast properties of the medium. The course of the reaction is affected by concentration of agar, its electrolyte content, thickness of the layer, placement of the hollows, pH, temperature of the reaction [13]. The reaction may be carried out at room temperature, at 36.5° and in the cold (at 4-6°). Temperature does not affect the distribution of precipitation lines. Addition of NaCl to the agar is necessary to prevent nonspecific precipitation even though the reaction proceeds more smoothly in the absence of salt [13].

The optimal conditions are: temperature 36.5°, pH 7.5 and sodium chloride concentration 0.8% [13]. It is very important for the success of the reaction that the antiserum have a high precipitation titer. In order to prevent rapid evaporation of the antigens and sera it is recommended that the dishes be covered and sealed hermetically by putting gauze dipped in molten paraffin around their edges; this obviates the need for adding ingredients to the hollows during the experiments.

In Ouchterlony's experiments precipitation lines appeared on the 7th-10th day [6]. In our experiments the agar concentration was lowered to 1% and concentrated antisera were used. This enabled us to observe the

appearance of precipitation lines at room temperature 18-20 hours after the beginning of the experiment.

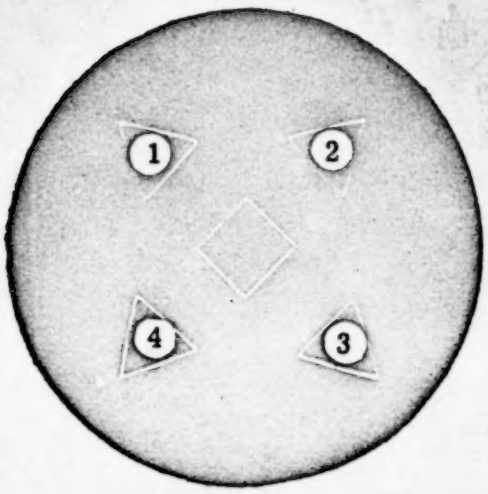

Fig. 1. Photograph of an experiment on specific precipitation reaction in agar with intracellular antigens. In the hollows: in the center – spleen cytoplasm antigen; in 1-4 – antiserum to whole tissue of leukemic spleen before and after depletion by nuclear antigen.

Halbert et al [2] used 0.6% agar and carried out the reaction at 4°. The results were available after 3 days. Jennings and Malone [3, 4] developed a more rapid method of precipitation reaction giving results in 2-4 hours.

Analysis of the reaction. When antigens diffuse against antiserum in agar, limited zones are formed in which the antigens and antibodies are found in optimal concentrations. Precipitation lines form in these zones. Figure 2, a, presents three variants of the reaction on comparison of various antigens. If the antigens being compared are identical, the precipitation lines cross; if only partial fusion of precipitation lines occurs with the formation of an appendage ("spur") it indicates that the antigens are related but are not identical [6, 7]. Reaction of this sort takes place in those cases in which the molecule of one antigen being compared is a carrier of several active groups whereas the molecule of the other one carries only some of these active groups.

The experiment on diffusion of hen and duck egg albumins against hen egg albumin serum is usually cited as an example of this reaction. This experiment is demonstrated in Fig. 2, b. Hen and duck egg albumins are related but not identical: antiserum to hen egg albumin reveals that two active groups are present in it, and only one of them in duck egg albumin. A fused precipitate line is formed by virtue of the active group common to both, while the appendage to this line indicates the incomplete similarity of the antigens being compared. Ouchterlony [7] called such a reaction "reaction of incomplete identity". This name has been retained although Oudin [11] criticized it on the grounds that identity excludes all differences and the name "reaction of incomplete identity" was incorrect.

It should also be pointed out that the name "identical reaction" is relative to some extent: formation of an arc-like fused precipitation line does not necessarily indicate that two antigens being compared are identical; it may be connected with the absence in the antiserum used of antibodies capable of demonstrating their differences. It is therefore essential to use antisera to both antigens when two antigens are being identified by specific precipitation reaction.

When two antigenic complexes are compared a number of precipitation lines is formed corresponding to the number of separate antigens taking part in the reaction. By removing a particular antigen from the complex or depletion by it of the antiserum it is possible to exclude that particular precipitation line and find out with which antigen-antibody system it is associated. As can be seen from Fig. 2, c, removal of antigen B from the complex led to the disappearance of the middle precipitation line, whereas removal of antigen A led to the disappearance of the line nearest to the antiserum reservoir. This permits the conclusion that the latter line is associated with antigen A and antibody anti-A, and the middle line with antigen B and antibody anti-B. Other methods have been proposed for identification of antigens in complexes by suppression of one or other precipitation line; these methods include depletion in the medium (addition of excess antigen to the agar), addition of excess antigen to the serum reservoirs etc. [1].

The distribution of precipitation lines with respect to the diffusion centers depends on the initial concentration of the ingredients, the rate of diffusion, the time needed for the formation of visible precipitate and on the solubility of the antigen-antibody complex. Depending on the concentration of antigens the points of intersection or fusion of precipitation lines dispose themselves either symmetrically about the antigen reservoirs or are displaced away from the reservoir containing excess antigen (Fig. 3, a).

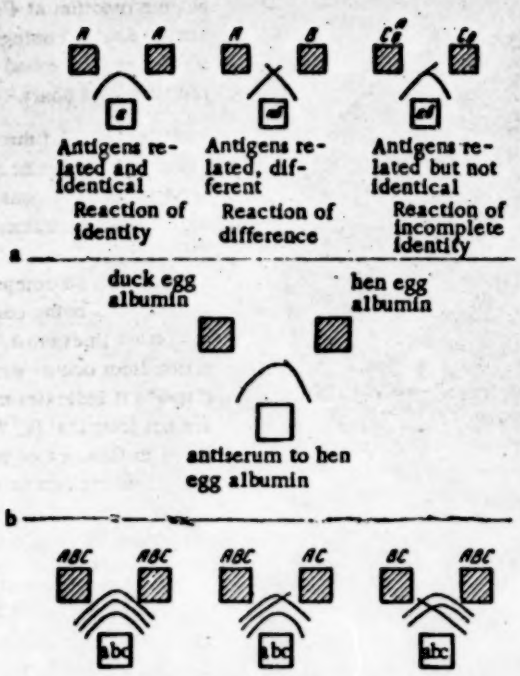

Fig. 2. Schemes of specific precipitation reaction in agar with simple and complex antigens.
a) Comparison of two antigens; b) example of reaction of incomplete identity; c) comparison of two antigenic complexes. Antigens are present in the shaded squares, antisera in the white ones.

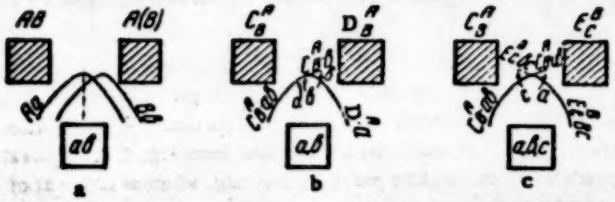

Fig. 3. Specific precipitation reaction in agar.
a) Comparison of two antigenic complexes. Precipitation line Bb displaced away from left reservoir in which antigen B is present in excess; b) comparison of a complex and a simple antigen with one common active group; one "spur" can be seen; c) comparison of two complex antigens with one common active group; two "spurs" can be seen. Antigens are in shaded squares, antisera to them in white squares.

Note. The scheme is based on the assumption that anti-A, anti-B and anti-C antibodies are independent molecules.

In model experiments with purified egg albumins Ouchterlony [7] determined more precisely the relation of the type of reaction to the nature and concentration of antigens. These experiments again confirmed that the appearance of multiple lines indicated multiplicity of antigens in a complex and that formation of appendages ("spurs") was connected with the fact that the composition of the molecule of one of the antigens included two active groups each evoking the formation of an independent antibody, while the molecule of the other antigen contained only one of these groups.

Investigation of mixture of homologous and heterologous antigens in various concentrations showed Ouchterlony that the reaction depended, to a certain extent, on the ratio of concentrations of the separate components of the mixture. Fusion of precipitation lines could occur in the presence of certain ratios. This is observed extremely rarely and simple dilution leads to separation of the lines and elucidation of the structure of the mixture or complexes.

"Pseudo spurs" may be formed when there is an excess of antigens. These, however, unlike the true ones, quickly dissolve and disappear. Nonetheless suitable concentrations of antigens must be chosen so that the precipitation lines would be symmetrically disposed, and trial runs with different concentrations are indicated.

Korngold [5] makes a detailed analysis of conditions under which true "spurs" are formed:

1) spurs are not formed when the antiserum contains only antibodies to those groups which are common to the antigens;

2) one spur is formed when the antiserum contains antibodies to both the group common to the two antigens and to the active group pertaining only to the homologous antigen (Fig. 3, b);

3) a double spur is formed in those cases in which the antiserum reacts with two antigens each of which has a common and a different active group and contains antibodies to all the active groups (Fig. 3, c).

In differentiating double "spurs" from "reaction of difference" it is essential to examine the nature of the appendages: the spurs are less solid than the main precipitation line, the change occurring abruptly; in the case of intersecting precipitation lines the decrease in solidity is gradual in the direction of the ends. Moreover, the precipitation lines fuse before the formation of "spurs" and remain "fused" after the formation of "spurs".

Differentiation of Antigen Complexes by Precipitation Reaction in Agar

Antiserum	Depletion by antigen	Test of depleted serum			
		Antigen	Reaction	Antigen	Reaction
Anti-(A+B)	B	B	Negative	A + B	Positive
Anti-C	C_B	C_B	"	C_B^A	"

The method of specific precipitation in a gel permits not only direct comparison of two antigens, determination of antigenic spectrum in an antigenic complex, identification of definite antigens in a complex, but also differentiation of complexes of independent antigens from complex antigens consisting of molecules with several active groups each of which is capable of evoking the formation of independent specific antibodies. This constitutes an important advantage of the given method over other serologic methods used for differentiation of antigens in antigenic complexes. For example, if the structure of antigenic preparations AB and C_B^A is studied by agglutination reaction then, as can be seen from the table, using appropriate sera and with their depletion by antigens B and C_B respectively the final reaction with the initial antigens will be the same in the case of the complex and the "mixed" antigen. In both cases the reaction demonstrates the presence in the serum of two antibody molecules with different specificities, but does not provide evidence on which it could be decided whether they are evoked by two separate antigens or by one antigen with two determinant groups; specific precipitation reaction, however, makes it possible to resolve this problem.

The method of specific precipitation in agar is simple, inexpensive and provides a practical means of differentiating any number of antigen components; it is almost as sensitive as other serologic methods. Very promising is the combination of this method with immunoelectrophoresis [12] which gives an electrophoretic characterization of each antigen participating in the formation of the precipitation line in the gel.

The method of specific precipitation in the gel has been used extensively in recent years for the study of problems in infectious and noninfectious immunology. It was used successfully (alone and in combination with immunoelectrophoresis) in the studies on the antigen structure of various infectious agents and products of their vital functions (staphylococci, streptococci, meningococci, tularemia and diphtheria bacilli, anerobic bacteria and their toxins, Hemophilus influenzae, small-pox virus, pig plague virus etc.), as well as in studies of antigenic structure of various proteins (snake venom, egg albumins, serum globulins in the normal and in cases of myeloma), antigens of normal and tumor cells in animals and man and antibodies in allergic and other diseases.

SUMMARY

Reaction of specific precipitation in agar is one of the methods of immunological analysis which have future prospects.

This method provides an opportunity to reveal the structure of complex combined antigens, to identify their separate components and to conduct a direct comparative analysis of various antigenic preparations. Special advantage of this method over other serological methods lies in the fact that it permits differentiation of the complex antigen, which is a molecule with two determinative groups from the combination or mixture of two antigens, each one of which represents a separate molecule.

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RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Ekspt. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratorii	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov,	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirurgii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vyshei Nerv. Deiatel.	Zhurnal Vyshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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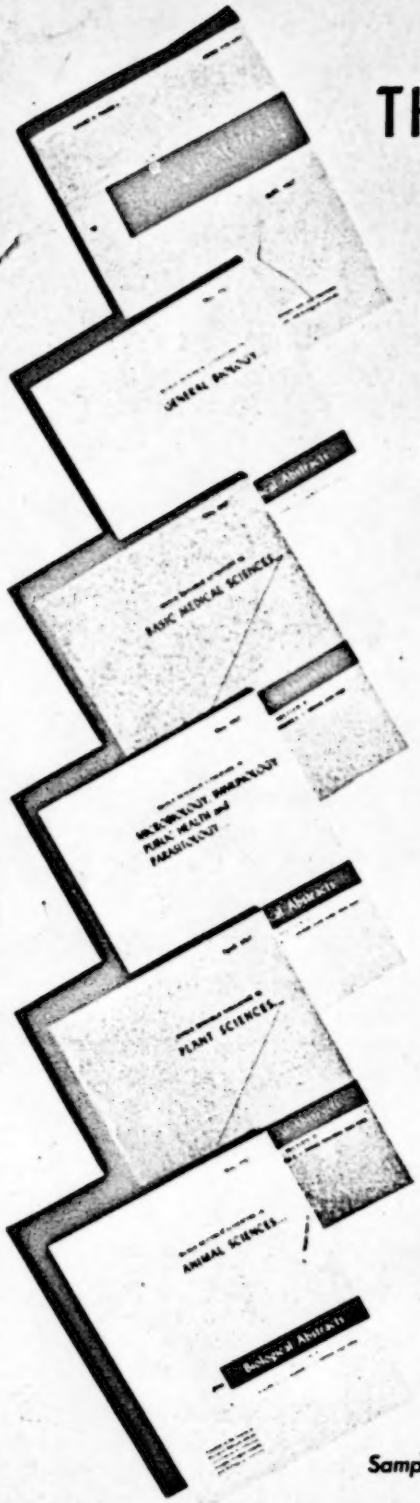

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